NCE LIBRARY

Volume LXX Number 2

THE BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

Editorial Board

GARY N. CALKINS, Columbia University
E. G. CONKLIN, Princeton University
E. N. HARVEY, Princeton University
SELIG HECHT, Columbia University
LEIGH HOADLEY, Harvard University
M. H. JACOBS, University of Pennsylvania
H. S. JENNINGS, Johns Hopkins University
E. E. JUST, Howard University
FRANK R. LILLIE, University of Chicago
CARL R. MOORE, University of Chicago
GEORGE T. MOORE, Missouri Botanical Garden
T. H. MORGAN, California Institute of Technology
G. H. PARKER, Harvard University
W. M. WHEELER, Harvard University
EDMUND B. WILSON, Columbia University

ALFRED C. REDFIELD, Harvard University
Managing Editor

APRIL, 1936

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

THE BIOLOGICAL BULLETIN

THE BIOLOGICAL BULLETIN is issued six times a year. Single numbers, $1.75. Subscription per volume (3 numbers), $4.50.

Subscriptions and other matter should be addressed to the Biological Bulletin, Prince and Lemon Streets, Lancaster, Pa. Agent for Great Britain: Wheldon & Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W.C. 2.

Communications relative to manuscripts should be sent to the Managing Editor, Marine Biological Laboratory, Woods Hole, Mass., between June 1 and October 1 and to the Institute of Biology, Divinity Avenue, Cambridge, Mass., during the remainder of the year.

INSTRUCTIONS TO AUTHORS

Preparation of Manuscript. In addition to the text matter, manuscripts should include a running page head of not more than thirty-five letters. Footnotes, tables, and legends for figures should be typed on separate sheets.

Preparation of Figures. The dimensions of the printed page (4⅜ x 7 inches) should be borne in mind in preparing figures for publication. Drawings and photographs, as well as any lettering upon them, should be large enough to remain clear and legible upon reduction to page size. Illustrations should be planned for sufficient reduction to permit legends to be set below them. In so far as possible, explanatory matter should be included in the legends, not lettered on the figures. Statements of magnification should take into account the amount of reduction necessary. Figures will be reproduced as line cuts or halftones. Figures intended for reproduction as line cuts should be drawn in India ink on white paper or blue-lined coördinate paper. Blue ink will not show in reproduction, so that all guide lines, letters, etc. must be in India ink. Figures intended for reproduction as halftone plates should be grouped with as little waste space as possible. Drawings and lettering for plates should be made directly on heavy Bristol board, not pasted on, as the outlines of pasted letters or drawings appear in the reproduction unless removed by an expensive process. Methods of reproduction not regularly employed by the Biological Bulletin will be used only at the author's expense. The originals of illustrations will not be returned except by special request.

Directions for Mailing. Manuscripts and illustrations should be packed flat between stiff cardboards. Large charts and graphs may be rolled and sent in a mailing tube.

Reprints. Authors will be furnished, free of charge, one hundred reprints without covers. Additional copies may be obtained at cost.

Proof. Page proof will be furnished only upon special request. When cross-references are made in the text, the material referred to should be marked clearly on the galley proof in order that the proper page numbers may be supplied.

Entered October 10, 1902, at Lancaster, Pa., as second-class matter under Act of Congress of July 16, 1894.

Vol. LXX, No. 2 April, 1936

THE
BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

CRUSTACEAN EYE-STALK HORMONE AND RETINAL PIGMENT MIGRATION

L. H. KLEINHOLZ

(From the Marine Biological Laboratory, Woods Hole, and the Biological Laboratories, Harvard University)

Introduction

The study of color changes in crustaceans has for a long time occupied the efforts of investigators. Pouchet (1876) believed such changes to be under the control of the nervous system. But Perkins' (1928) and Koller's (1928) independent discoveries that a hormone, which effected concentration of the body pigment, was elaborated in the eye-stalks of *Palæmonetes* and of *Crangon* opened a fertile field for research. Since that time a series of studies by Koller and Meyer (1930), Smith (1930), Kropp and Perkins (1933), Hosoi (1934) and Hanström (1935) has shown this hormone to be present in a large number of crustaceans.

Brown (1935) has offered the opinion that more than one chromatophore-activating hormone is secreted from the eye-stalk into the blood stream. Hosoi's report (1934) that extracts of various organs of *Penæus japonicus* were able to concentrate the chromatophores of blinded *Paratya compressa*, might be taken to indicate that the chromatophore reaction is merely a non-specific reaction to protein extract. But I am inclined to attribute his results to traces of the hormone present in the blood coagulated within the various organs used.

This heretofore entirely physiological problem was given a morphological aspect by the discovery in crustacean eye-stalks of certain structures which seem to be secretory in character (Koller, 1930; Hanström, 1931, 1935; Sjögren, 1934). Brown (1933) and Hosoi (1934) reported the presence of the chromatophore activator in extracts of the ventral nerve cord, but no histological study of this organ has been made to discover similar secretory structures.

The complexity and similarity of this eye-stalk hormone to the pituitary secretions of vertebrates had already suggested itself to Kropp

(1932), who investigated the possibility of a gonadotropic effect on immature rats. In 1934 Navez and Kropp found that the crustacean chromatophore activator possessed growth-promoting properties when tested by means of decapitated coleoptiles of *Avena*.

Closely related to this subject of control of crustacean body pigments has been that of retinal pigment migration. The literature, up to within recent years, deals almost exclusively with such topics as the effects of light intensities, spectral colors, and temperature on the rate of migration. Only recently (Bennitt and Merrick, 1932) has there been any attempt to approach this problem from a chemical basis.

Parker (1897), in his study of the structure and positional changes of the retinal pigment cells in *Palæmonetes*, could find no nerves supplying the distal pigment cells. Still, the problem of interrelationship of the eyes, a controversial and as yet unsettled one in vertebrate physiology, was brought into the discussion. The crux of the problem, briefly, is this: that if retinal pigment migration is independent of the central nervous system, then the condition of the pigment in the light-adapted eye of an animal should have no effect on that of the other covered eye of the same animal, and, conversely, if the central nervous system is involved in retinal pigment migration, one eye may be "sympathetically" influenced by the condition of the other eye. The evidence presented on this point has been inconclusive. Parker (1897) believed from his experiments with *Palæmonetes* that retinal pigment migration was independent of the central nervous system, but von Frisch (1908) using *Palæmon* could get no decisive results. Castle (1927), who confined his study to the proximal pigment cells in the eyes of *Palæmonetes*, concluded that an illuminated eye was without "sympathetic" effect on the position of the pigment in the covered eye. Bennitt (1924, 1932*a*) worked with several decapod crustaceans and found that the condition of one eye influenced the other covered eye of the same animal.

In 1932 Parker suggested the possibility of a hormonal agent in retinal pigment migration, a factor already hinted at by Welsh (1930*b*) and by Bennitt. This field of research was suggested to me by Professor G. H. Parker, to whom I wish to express my thanks and obligations for many kindnesses. The specific problem undertaken was to investigate the action of the crustacean eye-stalk hormone on the retinal pigments.

Material and Methods

The extracts of the eye-stalks of the various animals used in these experiments were prepared in essentially the same manner. A number of stalks were excised at their proximal ends and were triturated by

means of a glass rod in a small Syracuse dish, the bottom of which had been previously roughened with powdered carborundum. The solvent was then added to this crushed tissue. Sea water was first used for this purpose, but was later abandoned in favor of amphibian Ringer's solution, since the latter was more constant in composition, being free from any dissolved foreign substances that might have occurred in the sea water, and could be used as a standard control substance.

After this preliminary treatment, the mixture was stirred for two minutes, as much of the coarse detritus separated off as possible, and the suspension of tissue in solvent drawn up into a hypodermic syringe. The extract was not filtered because of the small quantities prepared at one time. Control extracts of other tissues of the animals were prepared in the same way, or ordinary Ringer's solution with no tissue was used.

In those experiments where the extract was to be prepared from eyes adapted to darkness, use was made of two adjoining dark-rooms. The specimens were kept in one dark-room and were removed singly to the second room where, with the aid of a photographic safelight (Brownie Series No. 1) both eyes were excised and immediately crushed. With practice this operation could be performed within 30 seconds. It seems reasonable to assume that this short period of exposure to dim light would not induce in the eye the formation of a substance which might affect the retinal pigment. When a sufficient number of eye-stalks had thus been accumulated and crushed, the solvent was added and the process of extraction continued as above.

Injections of these extracts were made into the appropriate animals. In the first tests where extracts were injected into light-adapted shrimps little difficulty was encountered, but in later experiments the necessity of injecting in the dark-room into dark-adapted specimens presented certain technical difficulties. The initial results, disconcerting in their negativeness, were attributable to such factors as difficulty in capturing and removing an animal from the aquarium, penetration of the hypodermic needle too far into the shrimp with the result that the injected fluid entered the gill space, and injection of too large quantities of extract thereby causing the death of the specimen. With progress of the work, however, a satisfactory technique was evolved, which is considered of sufficient value to be described here in detail.

Before starting the experimental animals adapting to darkness, each shrimp was placed into a 600 cc. beaker containing about 300 cc. of sea water. The beakers were then transported to the dark-room and arranged in linear groups of five. Ten to twenty-five animals could thus be used conveniently for a single experiment. After an interval suffi-

cient to assure dark-adaptation the specimens were ready for injection.

Parker (1897) found that the time required for complete outward migration of the distal pigment cells in adaptation to darkness was from 105 to 120 minutes, while Welsh's (1930*a*) observations indicate that the time for this migration is only slightly less, from 90 to 120 minutes. In the early part of this study shrimps were left in the dark-room usually overnight, but this was later shortened to a period of 5 hours. This interval also suffices for complete dark-adaptation of the proximal and reflecting pigments. Adaptation to light was obtained by keeping specimens in a white porcelain dish which was illuminated from above by an electric lamp.

Upon entering the dark-room, the first animal in the series was removed from its beaker and held in the left hand. After orienting the specimen with its dorsal side uppermost, the tip of the hypodermic syringe, which was held in the right hand, was inserted between two of the abdominal tergites. As soon as the needle was felt entering, it was twisted downward and slipped forward a fraction of an inch, so as to enter the abdominal musculature obliquely. The plunger was then tapped gently downward about half a dozen times, the needle withdrawn, and the shrimp replaced in its beaker. Immediate exit was made from the dark-room to record the time and amount of extract injected. This process was then repeated for the entire series of animals in an experiment. With a little practice, manipulation of the hypodermic plunger could be well controlled, with the consequence that, in the several hundreds of specimens used in this study, amounts of extract ranging from 0.01 cc. to 0.05 cc. were injected. Occasional difficulty was encountered when an animal jerked while injection was being made. In some such cases the needle might emerge and part of the extract would be lost. All cases of this sort where there was any doubt as to whether the extract had entered or remained in the specimen were noted. It may be added, parenthetically, that there could be no absolute certainty that the complete volume of injected extract remained within the animal; a small part might have been expelled by the body movements of the shrimp, but it is hoped that this possible error was reduced to a minimum by injecting obliquely forward.

In order to make readings of the position of the pigment in the eyes of the experimental specimens, two methods were used. In one (Welsh, 1930*a*), where the experimental animal was adapted to light, observations were made chiefly on the living shrimp. To this end the bottom of a Petri dish was covered with a layer of soft paraffin from which a block about one-half inch square was cut, exposing the glass bottom of the dish. A shrimp was fastened down by means of sta-

tioner's staples so that its eye-stalks projected over this opening and the dish was then filled with sea water sufficiently to cover the specimen. By placing the vessel on the stage of a microscope, the position of the distal retinal pigment could be observed and recorded by means of a micrometer eye-piece. After an injection of eye-stalk extract, the animal could again be fastened down and the position of the pigment measured at suitable intervals.

In the second method where the specimens were adapted to darkness before injection of the stalk extract was made, it was necessary to fix the eyes before any readings could be taken of the position of the pigment. This was done by killing animals at varying intervals after injection by immersion in hot water (80°–90° C.) for 10 to 15 seconds. Following this preliminary fixation, the eye-stalks were excised, dehydrated, and cleared, either in oil of cedarwood or in beechwood creosote. The position of the distal pigment in the cleared eyes could then be measured. It was necessary to embed and section the eyes to observe the proximal and reflecting pigments. This was facilitated by first washing the cleared eye-stalks with ether-alcohol solution and then embedding doubly in celloidin and in paraffin. Sections were cut at 10 micra, some being subsequently stained with Delafield's hematoxylin and eosin, and others being mounted unstained.

The animals used in this study were all crustaceans of the order Decapoda. The common marine shrimp, *Palæmonetes vulgaris,* was chosen as the test organism and, for a number of reasons, proved to be very favorable material. First, it is a commonly occurring form that is easily obtained in sufficient quantities. These specimens were readily secured in Boston from a dealer in live bait. During the summer months at Woods Hole they were caught by sweeping a dip-net along pilings and rocks covered with the alga, *Fucus.* Secondly, the mechanics and time periods for retinal pigment migration are well known through the excellent studies of Parker and of Welsh. Thirdly, the test shrimp, *Palæmonetes,* does not experience the retinal condition of persisting diurnal rhythm (personal communication from Dr. Welsh) which has been found occurring in a number of crustaceans (Welsh, 1930*b*, 1935; Bennitt, 1932*b*).

Animals whose eye-stalks were tested for the presence of a hormone that affected retinal pigment were: *Palæmonetes vulgaris,* the common shrimp; *Uca pugilator,* the fiddler crab; *Libinia dubia,* the spider crab; *Cancer irroratus,* the rock crab; *Carcinides mænas,* the green crab; and *Callinectes sapidus,* the blue crab. The results of this investigation are considered below under separate headings.

Structure of the Eye of Palæmonetes

For a detailed description of the finer structure of the eye and an account of the mechanics of pigment migration, the reader is referred to the papers of Parker (1891, 1897) and of Welsh (1930*a*, 1932). For this paper a description of the three types of pigment cells and their relation to the eye as a whole is deemed sufficient.

The eye of *Palæmonetes* is a compound structure consisting of ommatidial units. Composing or concerned with each ommatidium (Fig. 1) are the following structures: cornea (*C*), cone, rhabdome (*RH*), fenestrated basement membrane (*BM*), and the three types of pigment cells, the latter enriched by a varied terminology, but for purposes of simplicity and greater descriptiveness referred to here as distal (*DP*), proximal (*PP*), and reflecting (*RP*) pigment cells.

In each ommatidium there are two distal pigment cells which form a collar around the cone. These cells possess distal processes which extend to the cornea, and proximal processes which appear to be continuations of the proximal pigment cells.

There are eight proximal cells, one of which is rudimentary, to each ommatidium, all surrounding the rhabdome. Proximally beyond the rhabdome these cells become attenuated, pass through the fenestrated basement membrane, and, as retinal nerve fibers, connect with the first optic ganglion. These proximal cells appear to be the only retinal cells to have an anatomical connection with the central nervous system.

The reflecting pigment cells are located near the basement membrane. Processes from them may extend proximally through the basement membrane as far as the first optic ganglion, while distal processes may reach outward to the tip of the distal retinal cells where some of the reflecting pigment is accumulated in a small cap. The number of reflecting cells is difficult to determine unless sections are treated by special histological methods to remove the pigment and stain the nuclei, but Parker (1897) believes that there are no more than one or two of these cells for each ommatidium.

Black pigment granules are contained in both the distal and the proximal retinal cells. In the reflecting cells the pigment is of a different sort. When this pigment is viewed through the microscope by transmitted light, it appears yellowish or greenish brown and is barely distinguishable from the black pigment of the proximal and distal cells; examination of the same section by dark-field illumination, however, shows the reflecting pigment as a gleaming white granular mass. The pigment itself is probably guanin, though, as far as I am aware, few studies of the chemical nature of this white pigment have been made among the

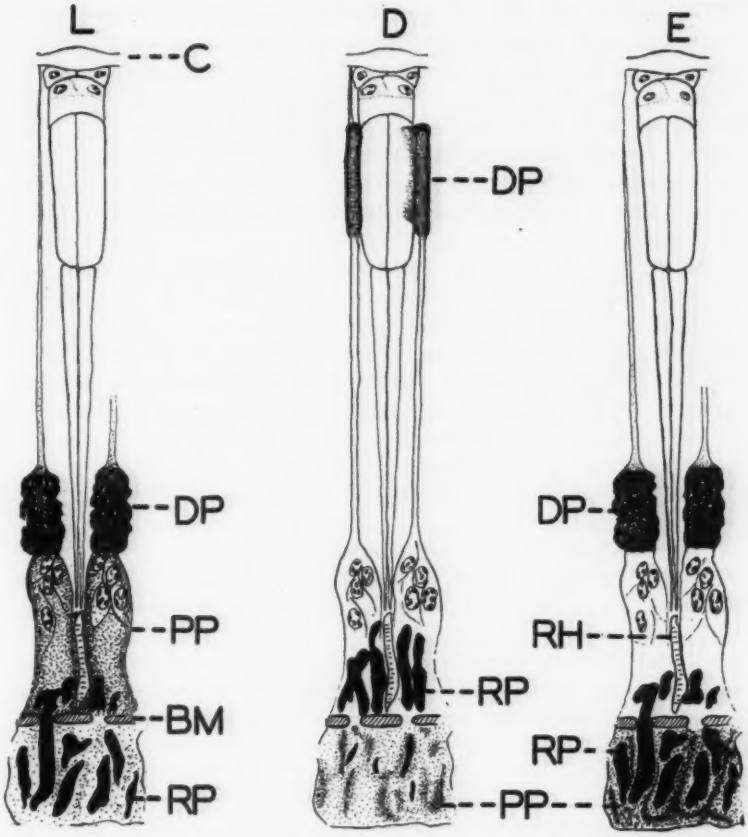


FIG. 1. Ommatidia from the eyes of *Palæmonetes vulgaris*, showing the general structure and the position of the three pigments under various conditions. *L*, from an eye in the light condition; *D*, from a dark-adapted eye; *E*, from an experimental animal which, after being adapted to darkness, was injected with stalk extract prepared from the eyes of light-adapted specimens. *C*, cornea; *DP*, distal pigment; *PP*, proximal pigment; *BM*, basement membrane; *RP*, reflecting pigment; *RH*, rhabdome.

invertebrates and none among the crustaceans. Guanin is commonly found occurring in crystalline condition in the integument of lower vertebrates (Ewald and Krukenberg, 1882, 1883). It is thought by some investigators to be formed from alimentary nucleoproteids and by others to be formed endogenously from nucleoproteids of the body tissues (Millot, 1922, 1923*a*, 1923*b*).

Normal Photomechanical Changes in the Eye of Palæmonetes

The positions of the retinal pigment cells characteristic for the conditions in darkness and in light are shown in Fig. 1. The distal pigment cells in eyes which have become fully adapted to darkness have migrated distally so that they terminate approximately at the outward level of the cones. In this position, according to Exner (1891), lateral rays of light might be admitted through one cone and then be allowed to diffuse to the receptors of adjacent ommatidia, thereby increasing the efficiency of the eye.

In the light condition, the distal pigment cells have migrated proximally a distance of 150–200 micra, and have the form described by Herrick (1891) of a "plaited black ribbon." Parker (1897) regarded this appearance as abnormal, due to a prolonged stay in the dark before exposure to light, but Welsh (1930*a*) has found this to be a normal occurrence. I find this same condition to be typical in my preparations of light-adapted eyes. In this position the distal pigment cells prevent overstimulation of the rhabdome by excluding lateral rays and by admitting only those axial light-rays which enter the rhabdome by way of the cone.

The proximal pigment may be considered as moving in directions opposite to that taken by the distal pigment in response to light and to darkness. In the light, the pigment granules are found in a distal position above the basement membrane, while in a dark-adapted eye the pigment has migrated proximally below the basement membrane. The means by which the migration of the proximal pigment occurs has not been studied, but it has been assumed by Parker (1897), Mossler (1915), and Bennitt (1924) to result from some form of protoplasmic movement.

Exner (1891) first called the layer of reflecting pigment the tapetum. Its function appears to be the reflection of light from within the eye so that in darkness or in dim light, rays, which have entered the eye and have passed through the rhabdome, may be reflected back again into the rhabdome or even out of the eye. Stimulation may thus be accomplished by repeated application of weak light to the rhabdome, where ordinarily the light would have been of insufficient intensity to have any retinal effect. The glow which emanates from the eyes of many crustaceans which have been kept for some time in the dark is due to the reflection of light from the eye by the reflecting pigment.

It was believed for a time that the reflecting pigment did not change its position under various conditions of light, and from the common understanding that this pigment acted solely as a reflector no photomechanical change was considered necessary, so long as the proximal

pigment moved to cover or uncover the tapetum. But Parker (1897) thought that the reflecting pigment in *Palæmonetes* underwent positional changes in adaptation to light and to darkness. This was supported by reports of Trojan (1913) and of Mossler (1915) on *Palæmon*, and confirmed and extended by Welsh on *Palæmonetes* and on *Macrobrachium* (1932). In the dark-adapted eye the greater part of the reflecting pigment lies above the basement membrane (Figs. 1, 5, 9, and 10), while in a fully light-adapted retina much of it, except for a small portion, lies in processes below the basement membrane (Figs. 1, 6, 11, 12). Little is known about the mechanics of migration of this pigment.

Palæmonetes vulgaris

Four groups of tests involving the use of eye-stalk extract from this animal were carried out. In the first two groups, observations were made on living specimens in the manner already described above;

TABLE I

Proximal migration of the distal retinal pigment cells in a series of dark-adapted test *Palæmonetes* as a result of injecting individuals with 0.02 to 0.04 cc. of an extract of 60 eyes of light-adapted *Palæmonetes* in 1.0 cc. of sea water. Specimens were killed at various intervals after injection. The position of the pigment is given in ocular micrometer units (1 unit being equal to 16.2 micra) and was measured from the surface of the cornea to the distal margin of the pigment cells.

Minutes after injection	Each eye		Minutes after injection	Each eye	
0	4.0	3.5	25	13.0	11.0
0	3.0	3.5	28	14.0	17.0
0	5.0	4.5	30	15.0	15.0
5	5.0	6.0	32	17.0	16.0
10	6.0	6.0	34	13.0	12.0
15	9.0	6.5	36	19.0	19.0
18	12.0	10.5	38	16.0	16.5
20	8.0	12.0	45	15.0	20.0
21	11.0	11.5	50	19.0	18.0
22	15.0	17.0	55	18.0	19.5

in the last two, the eyes of the test shrimps were fixed, dehydrated, and cleared before measuring the migration of the distal retinal pigment.

In an attempt to determine whether the crustacean eye-stalk hormone would cause an elongation of the distal pigment cells in light-adapted shrimps, suitable specimens were injected with two kinds of extracts. Some received extract prepared from the stalks of shrimps that had been in the dark-room overnight, while others were treated with stalk extract from the eyes of animals which had been adapted to a black background. In neither of these cases was there any significant change in the position of the distal pigment cells (Kleinholz, 1934).

In the third group of tests, the entire procedure was reversed in order to see whether the eye-stalk hormone would induce a contraction

of the distal retinal pigment similar to that produced on the body pigment. Stalk extracts were prepared from the eyes of light-adapted specimens and were injected, in the dark, into test shrimps which had

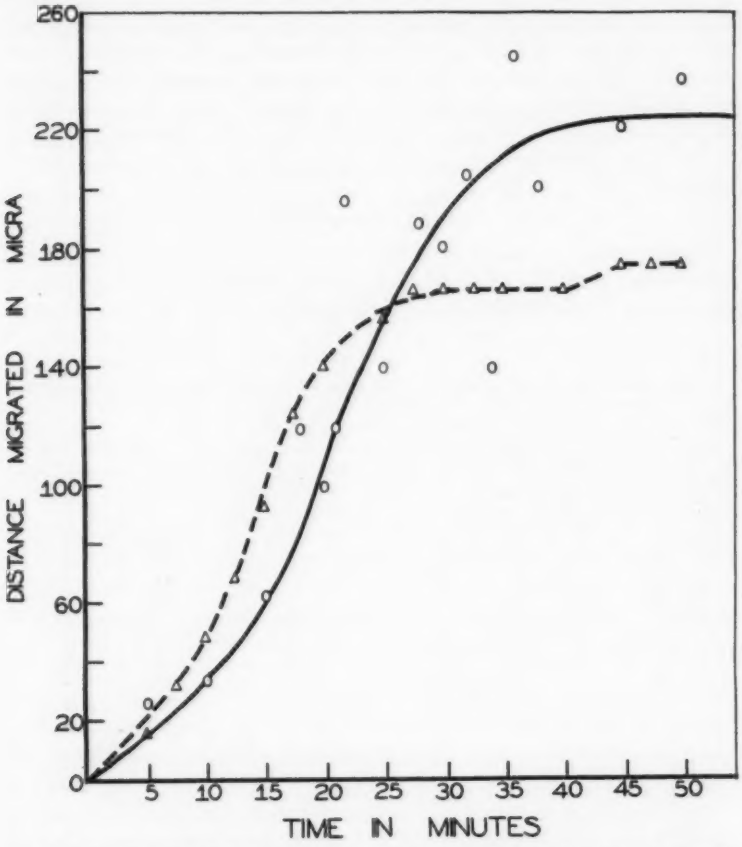


FIG. 2. Results of the measurements shown in Table I, plotted to show rate of migration of the distal pigment cells in a series of animals which have been injected, while adapted to darkness, with extract from the eye-stalks of light-adapted *Palæmonetes*. The broken curve represents Welsh's figures on the rate of migration of the distal pigment during adaptation to light.

been adapting to darkness overnight. An extract of 60 stalks in 1.0 cc. of sea water was prepared and injected in doses ranging from 0.02–0.04 cc. into the animals of a series. After the preliminary fixing and clear-

ing of the stalks of the injected shrimps, the position of the distal retinal pigment was measured by means of a micrometer eye-piece. Proximal migration of this pigment had occurred, a maximum being reached in those animals killed from 30–45 minutes after injection.

The set of measurements for one such series is represented in Table I. The three uninjected controls were killed at intervals during the course of the experiment, one when injections into the test shrimps were about to be started, one at about the middle of the experimental period, and the third when the last injected specimen was fixed. Since the controls showed some variation in the position of the pigment, not only among themselves, but also between both eyes of the same animal, the average position of the distal pigment in all six eyes was taken as the "zero reading" for dark-adaptation.

The average measurements from both eyes of the test specimens of this series, when converted into micra and plotted against time after injection, give the curve (solid line) shown in Fig. 2. The broken-line curve in this figure is a transcription of Welsh's measurements on the rate of migration of the distal pigment cells during the course of adaptation to light. The general similarity of the two curves is at once evident. The rate of change is slow at first, but after 10 minutes is increasing rapidly, being 10 micra per minute for Welsh's curve, and about 6.5 micra per minute for mine. Welsh's figures show that the rate of migration begins to decline after about thirty minutes when the migration has neared its maximum, but that there is a secondary movement when the distal pigment cells contract into the plaited form observed by Herrick (1891). Since my measurements were made on a number of animals and are consequently subject to more variation than Welsh's consecutive readings on the same individual, I have not felt justified in indicating this secondary movement graphically, although from a study of my sectioned material it is known to occur.

The difference in rate of migration between the two curves invites interesting speculation. If we assume that in the dark no effective hormone is being released into the blood stream and that with light stimulation such a hormone is secreted until the pigment has contracted, we may expect that constant stimulation of the eye by light will result in a continuous secretion of the hormone to maintain the pigment in a state of contraction. Since my test specimens, however, were kept in the dark-room throughout the course of the experiment, the only hormone present within the body was that injected into the shrimps. The dosage and concentration used makes this equivalent to 1.5 light-adapted eyestalks injected into each individual. The experimentally injected animals thus not only had a smaller amount of hormone than light-adapting

shrimps, but were also lacking a continued secretion which would occur during the process of adaptation to light. At this time, however, not very much importance can be attached to this aspect of the problem. More exact quantitative treatment must be delayed until the crustacean eye-stalk secretion can be isolated or prepared in purer form.

The scatter and the greater amount of migration obtained in my curve is due, I think, to variations in size of individual eyes and to the fact that my specimens were probably slightly larger than those used by Welsh.

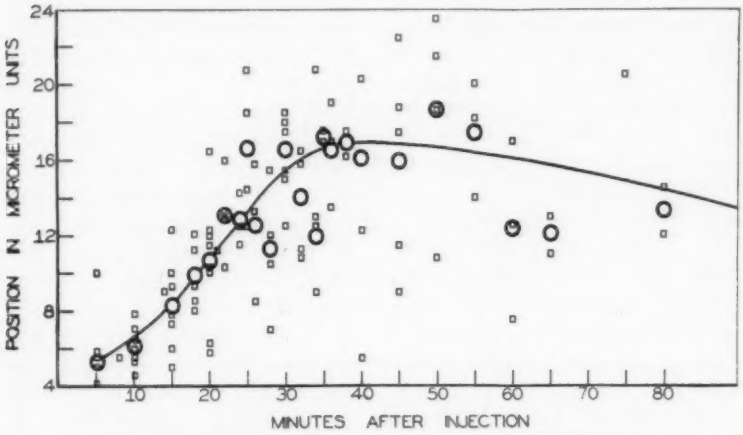


FIG. 3. Graph showing the change in position of the distal pigment in the eyes of dark-adapted *Palæmonetes* at varying intervals after injection with light-adapted eye-stalk extract. Each square represents the average of the positions in both eyes of a single animal, and the larger circles represent the averages of all eyes measured at similar intervals after injection. The wide scatter shows the inadequacy of this method for quantitative measurements.

Any attempt at an exact quantitative determination of rate of migration of the distal pigment, made by killing individuals at varying intervals, is necessarily unsatisfactory. For rough estimates, these methods, used by Parker (1897), Mossler (1915), and Welsh (1930*a*), indicate the time required for complete adaptation to light or to darkness. But even so there is a considerable degree of deviation. Welsh reports, " In some eyes the distal pigment cells would have reached the extreme position characteristic for the light in thirty minutes, and in other eyes this would not be reached until sixty or seventy minutes in the light."

Mossler (1915) attempted to reduce this error by taking measurements on a number of eyes after each period of exposure to light and

then averaging the results. But this too was not very satisfactory. I have attempted a similar though more extensive method, using measurements made on about 200 eyes from dark-adapted *Palæmonetes* which had been injected with extracts of light-adapted stalks. The results are shown in Fig. 3. In this graph each square represents the average of the position of the distal pigment in both eyes of an experimental animal; the large circles represent the averages for all specimens killed at the same periods after injection. Except for about half a dozen specimens which showed little or no response to treatment with the eye-stalk extract, possibly because of inadequate injections, practically all of the other experimental animals showed some degree of distal pigment migration, although here too there is some discrepancy, even in animals killed at similar times after injection. These variations are reasonably attributable to such factors as individual differences in the size of the eyes and in the physiological conditions of the animals. Welsh (1930*a*) came to the conclusion that no really accurate measurement of the rate of migration of the distal pigment could be made except by studying this process in single individuals. Similarly, it is my opinion that any further quantitative studies of hormonal effects on this process will need to be made by a method identical with or similar to that finally used by Welsh.

When sections of eyes from experimental animals in this third group of tests were examined histologically, the proximal migration of the distal pigment was confirmed. The proximal pigment was apparently unaffected by the stalk extract, since it remained in the position typical for darkness. The reflecting pigment, however, was observed to have migrated proximally into the light condition. In an earlier paper, Welsh (1935) mentioned some of my unpublished results where the eye-stalk hormone was reported to have no effect on the reflecting pigment. At that time I had examined the first of my preparations only superficially and was misled by the difficulty of distinguishing, by transmitted light, between the pigment in the proximal and in the reflecting cells. Subsequent examination of the same and additional material by means of dark-field illumination showed that after injection of stalk extract the greater part of the reflecting pigment had moved below the basement membrane (Figs. 1, 7, 8, 13, 14).

Two control experiments were performed for this third group of tests. Whenever a series of *Palæmonetes* was put into the dark-room in preparation for injection with stalk extract, several primary control specimens were included along with it. These primary controls were uninjected and were fixed at intervals during the course of each series. The distal pigment in the eyes of such controls showed variations of

1–3 micrometer units (16–48 micra). Three series of secondary controls consisted of specimens which were first adapted to darkness and then injected either with sea water, cold-blooded Ringer's solution, or sea-water extract of shrimp's abdominal musculature. A total of 47 specimens were treated as secondary controls and in none of these was there any momentous change in the position of the distal pigment cells. The slight variation noted in the primary controls was also evident here, but this was undoubtedly due to differences in the eyes of individuals and has no significance for these experiments.

In the fourth group of tests, shrimps were adapted to darkness as before, but the stalk extract was prepared from *Palæmonetes* which had been adapted to darkness. The method of preparing these dark-room extracts has been described above. Concentrations equivalent to 60 stalks in 1.0 cc. of amphibian Ringer's solution were injected in small quantities (0.02–0.04 cc.) into 41 dark-adapted test animals. The specimens were killed at an average time of 33 minutes after injection and the position of the distal pigment measured. A partial migration of the distal pigment towards the condition characteristic for light occurred, but this movement was not as marked as that resulting from the injection of extracts prepared from light-adapted stalks. For purposes of comparison the average position of the distal pigment in the eyes of this group are represented graphically with that of the third group where the same concentration of extract was injected into a nearly equal number of specimens (Fig. 4). Extracts from light-adapted eyes are seen to be almost twice as potent as those prepared from dark-adapted eyes. The probable significance of this difference will be discussed below.

Cancer irroratus

In examining the effects of stalk extracts from the eyes of various crustaceans on the retinal pigment of the test animal, *Palæmonetes*, the chief interest centered on the response of the distal pigment cells which were most easily observable, but where possible, the fixed eyes of the test shrimps were sectioned to study also the reactions of the proximal and reflecting pigments.

Since it became apparent from the early work with *Palæmonetes* extract that no reliable measurements could be obtained for the rate of response of the distal pigment to stalk extracts by the method used, it was decided to abandon this procedure, and, by a roughly quantitative method, attempt to study the effects on an "average" test specimen. Shrimps which survived treatment with a particular extract were killed at an interval of 30–40 minutes after injection. The average time the extract was allowed to act before killing the test animals and the average

TABLE II

Average time extract was allowed to act before killing animals and average position of distal pigment

Source of eyestalks	Number of animals injected	Number died	Average time killed after the injection	Number of eyes measured		Position of distal pigment		Average position of pigment	
				Injected	Control	Injected	Control	Injected	Control
			min.						
Cancer irroratus	46	0	33.8	91	16	24,121	1,360	265.0	85.0
Libinia dubia	46	4	35.0	84	20	28,204	1,636	335.7	81.8
Uca pugilator	31	2	36.4	58	26	14,337	2,300	247.2	88.3
Callinectes sapidus	66	13	31.4	106	18	10,935	1,425	102.8	79.2
Palæmonetes vulgaris (light-adapted)	30	0	32.6	60	14	14,742	810	245.7	57.8
Palæmonetes vulgaris (dark-adapted)	41	6	32.5	70	18	10,465	1,409	149.5	78.3
Carcinides mænas	34	6	38.0	56	19	11,485	1,506	205.1	79.3

position of the distal pigment for all the eyes studied in a series were then calculated and tabulated (Table II). The measurements obtained from the eyes of primary controls were treated in the same fashion. A number of secondary controls, consisting of test *Palæmonetes* into which various tissue extracts had been injected, were also studied, but these observations were not included in the tabulated results.

In this first series, 46 specimens of *Palæmonetes,* adapted to dark-

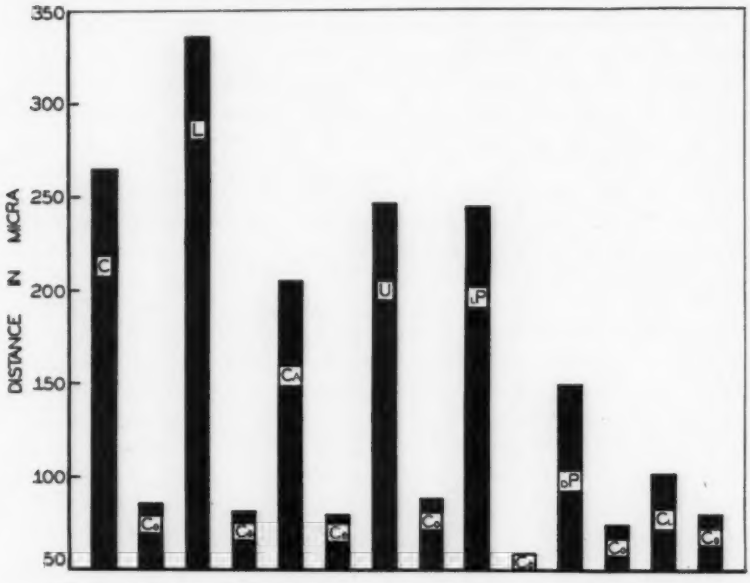
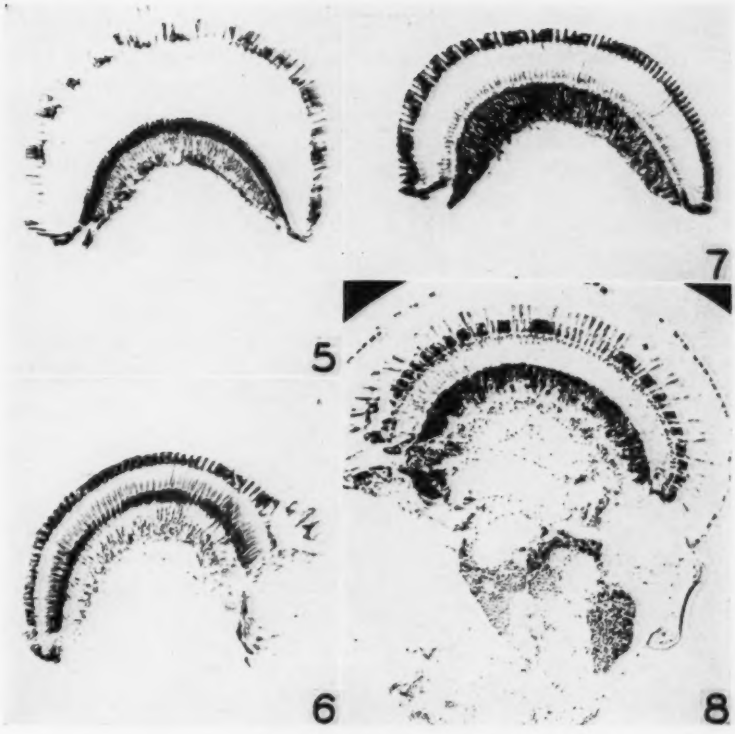


FIG. 4. Graphic representation of the position of the distal pigment in "average" test specimens as a result of injecting extracts from the eyes of various decapod crustaceans. *Co,* control uninjected specimens; *C,* extract from eye-stalks of *Cancer irroratus; L,* extract from eyes of *Libinia dubia; CA,* stalk extract from *Carcinides mænas; U,* extract from *Uca pugilator; LP,* extracts from eyes of light-adapted *Palæmonetes vulgaris; DP,* extracts from darkness-adapted eyes of *Palæmonetes vulgaris; CL,* extracts from eyes of *Callinectes sapidus.*

ness, were injected with small quantities (ranging from 0.02 to 0.04 cc.) of an extract of 10 light-adapted eye-stalks of *Cancer irroratus* in 0.8 cc. of amphibian Ringer's solution. The test animals were killed about 35 minutes after injection and the position of the distal pigment measured. The range of response was variable, as might have been expected from the earlier work. The lowest value (in one eye) was about 194 micra and the highest value (in one eye of another specimen) was



EXPLANATION OF PLATE I

FIG. 5. Longitudinal section of a normal retina of *Palæmonetes vulgaris* in the "dark" condition. Only portions of the distal pigment cells show, due to the plane of sectioning. The reflecting pigment appears as a dense black mass above the basement membrane which is clearly visible. Below the basement membrane is the more dispersed proximal pigment. At the very bottom of the section are traces of reflecting pigment which apparently never migrates completely above the basement membrane.

FIG. 6. Longitudinal section of a normal light-adapted retina of *Palæmonetes*. The distal pigment has migrated proximally. Immediately below the distal pigment is the diffuse proximal pigment which has migrated above the basement membrane. Below the proximal pigment is the mass of reflecting pigment from which many processes are seen extending below the basement membrane.

FIG. 7. Longitudinal section of a dark-adapted *Palæmonetes* retina. This specimen was injected, in the dark, with 0.02 cc. of an extract of 20 light-adapted *Palæmonetes* eye-stalks in 0.4 cc. of sea water. The retina was fixed 45 minutes after injection. The distal pigment has migrated proximally. The proximal pigment has remained below the basement membrane, while nearly all of the reflecting pigment has moved proximally below the basement membrane.

FIG. 8. Longitudinal section of the eye-stalk of a dark-adapted *Palæmonetes*. This specimen was injected, in the dark, with 0.03 cc. of extract of 4 light-adapted *Libinia dubia* eye-stalks in 0.4 cc. of amphibian Ringer's solution, and was killed 35 minutes after injection. Stained with Delafield's hematoxylin and eosin. The position of the three pigments is the same as in Fig. 7.

372 micra; both of these specimens were killed at the same interval after injection of almost equal amounts of the extract. The average positions of the distal pigment for the 46 experimental (265 micra) and the 16 primary control shrimps (85 micra) are shown graphically in Fig. 4. Six secondary controls were injected with 0.02–0.05 cc. of an extract of cheliped muscle in Ringer's solution. The average time specimens were killed after injection was 30.5 minutes and the average position of the distal pigment was 69.2 micra.

The eyes of two of the test animals were embedded and sectioned. The proximal pigment was seen to have remained in the position characteristic for darkness, below the basement membrane, but the reflecting pigment had, for the most part, moved below the basement membrane into a position typical for light-adapted eyes.

Libinia dubia

In this experiment 46 test *Palæmonetes* were injected with 0.01 cc. to 0.04 cc. of an extract of light-adapted eye-stalks from *Libinia dubia*. The concentration used, equivalent to 10 stalks in 1.0 cc. of Ringer's solution, was well tolerated by the shrimps, only 4 of them dying during the course of the experiment. Of the 42 surviving animals, two failed to show any response of the distal pigment, but all others showed a very marked contraction of the distal cells, in several specimens the position being measured at 437 micra from the margin of the cornea. The average position for the 42 surviving *Palæmonetes*, including the two which showed no response at all, was 335 micra, and that for the primary controls was 82 micra. Five secondary controls were injected with extract prepared from the maxillipeds of *Libinia*. The average time during which the extract was allowed to act was 32 minutes, and the average position of the distal pigment at the end of this time was 76 micra.

EXPLANATION OF PLATE II

These photographs were taken by means of a Zeiss cardioid condenser.

FIG. 9. The same section shown in Fig. 5. This print has been retouched to indicate the expanded position of the distal pigment cells, made evident by the small caps of reflecting pigment at the distal ends of these cells. The main mass of the reflecting pigment lies above the basement membrane.

FIG. 10. A high-power detail of a portion of Fig. 9, showing the pigment above the basement membrane and the small amount at the proximal limit of the retina.

FIG. 11. The same section as shown in Fig. 6. The contracted condition of the distal pigment cells is indicated by the caps of reflecting pigment on the cells.

FIG. 12. A portion of Fig. 11 in high-power view. The reflecting pigment is seen extending proximally in processes below the basement membrane.

FIG. 13. The same as the section in Fig. 7.

FIG. 14. An enlargement of a portion of Fig. 13, showing the large number of processes containing reflecting pigment, due to a proximal migration induced by injection of eye-stalk extract.

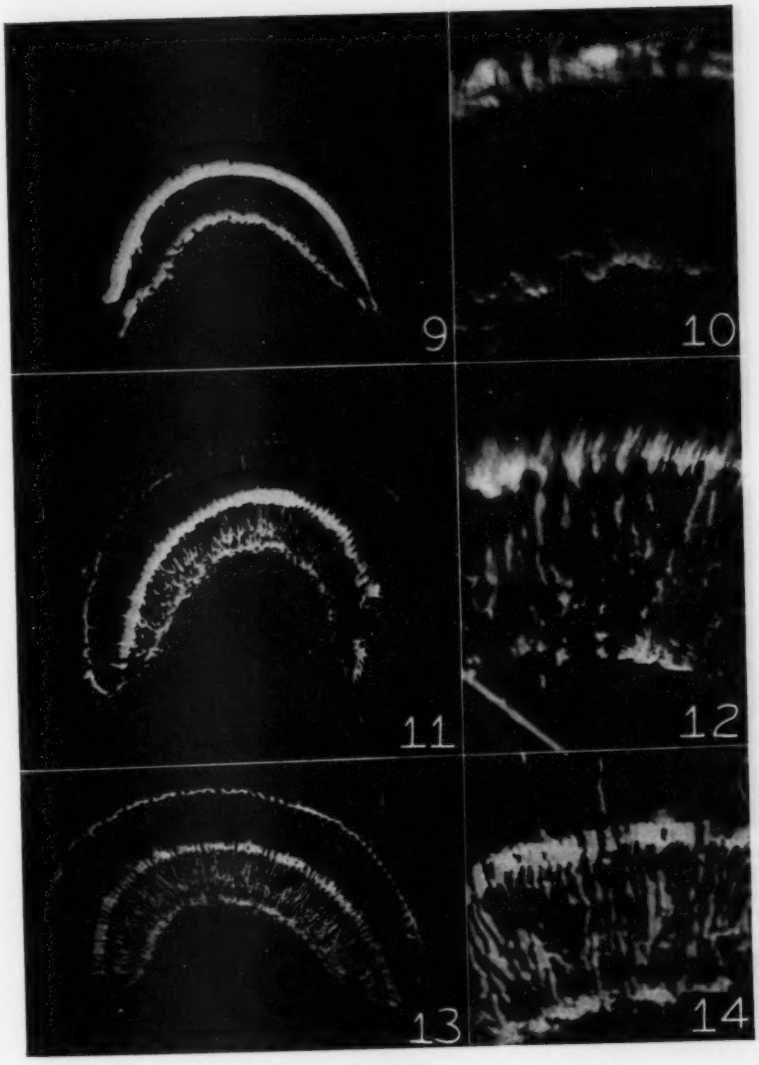


PLATE II

Sections of the eyes of three of the test *Palæmonetes* showed the proximal pigment persisting in the dark condition, but the reflecting pigment migrated into the light condition (Fig. 8).

Uca pugilator

Thirty-one test shrimps, which had been adapted to darkness, were injected with 0.02 cc. to 0.04 cc. of an extract of *Uca* eye-stalks, a concentration of 15 stalks to 1.0 cc. of Ringer's solution being used. Two of the test animals died during the course of the experiment, and only one of the 29 surviving *Palæmonetes* failed to show any change in position of the distal pigment; this exception was one that had been designated as doubtfully injected. In three eyes the position showing a small amount of migration was as low as 128 micra, but in the remaining eyes the range was up to a maximum of 356 micra. The average position for the test specimens was 247 micra, for the primary controls it was 88 micra, and for 5 secondary controls (cheliped muscle in Ringer's solution) the position was 68.0 micra.

The eyes of two of the *Palæmonetes* injected with extract from the stalks of *Uca* were sectioned. The proximal pigment was in the position characteristic for darkness, while the reflecting pigment showed a slight migration into the position characteristic for the light condition.

Carcinides mœnas

In a preliminary experiment, 12 test *Palæmonetes* were injected with small amounts of *Carcinides* eye-stalk extract, in a concentration of 10 stalks per 1.0 cc. of saline solvent. This proved to be too concentrated, resulting in the death of 5 of the injected shrimps. With a slightly diluted extract (equivalent to a bit less than 8 stalks in 1.0 cc. of amphibian Ringer's solution) the survival was much better, only one of the test specimens succumbing to the injection. Five of the injected animals showed only very slight evidence of change in position of the distal pigment (the reading being at about 130 micra), while the remaining shrimps showed responses that were marked but slightly lower than those obtained previously with extracts from other crustaceans (Fig. 4).

The average position of the distal pigment in 26 surviving test specimens was 205 micra, that for 10 uninjected primary controls was 79.3 micra, and for 6 secondary controls injected with extract of cheliped muscle, the position was 76.5 micra.

No eyes were sectioned for observation of the proximal and reflecting pigments.

Callinectes sapidus

In early tests, the injection of extract prepared from the eye-stalks of *Callinectes,* in a concentration of 2 stalks to 1.0 cc. of Ringer's solution, produced a very slight, almost doubtful response of the distal pigment. In an attempt to increase the magnitude of the reaction, extracts of higher concentration (5 stalks to 1.0 cc. of solvent) were prepared and injected. Unfortunately, in these cases the crude extract proved to be very toxic, killing many of the test *Palæmonetes.* Those animals which did survive the injection did not show any increased response over those treated with the more dilute extract. The average position of the distal pigment for 53 surviving test specimens was 102.8 micra, and that for 9 primary controls was 79.2 micra. No secondary controls were carried for this series, and none of the eyes were sectioned for observation of the other retinal pigments.

The results obtained by using extracts prepared from eye-stalks of *Callinectes* are difficult to understand. Perkins and Kropp (1932) and Hanström (1935) found that stalk extracts prepared from this crustacean were effective in concentrating the body pigment of *Palæmonetes;* the potency of my extracts was tested by injection into dark *Palæmonetes* and the typical contraction of the body pigment was obtained. The response of the shrimp's retinal pigment to this extract is so slight as to be almost within the limit of experimental error. Two possibilities suggest themselves in explanation of this situation. There may be a difference in concentration threshold between the retinal pigment and the body pigment, and it may be that my extracts were not sufficiently purified (by filtering) to allow the use of more concentrated preparations which would not prove toxic. Or else, it is possible that crustaceans possess several pigmentary hormones, and that in *Callinectes* the hormone affecting the retinal pigment is lacking. This point requires further experimental investigation.

Discussion

In studies of processes under hormonal control two general methods of procedure are open to the investigator. One is to remove the tissue thought to secrete the active agent, and the other is to inject extracts of the organs suspected of endocrine function.

Unfortunately, little use can as yet be made of the first method in studies of the crustacean pigmentary hormones, because there is a scarcity of information about the specific tissue within the eye-stalk which is involved in such activity. Only recently have there been any morphological studies of the crustacean eye that have revealed the pres-

ence of structures with a possible hormonal function. This has been reported by Hanström (1931, 1934) and one of his students, Sjögren (1934). These investigators found two different tissues, an X-organ and a "blood gland," which they think may play a part in the control of crustacean color changes. Professor Hanström has very kindly allowed me to read a preliminary paper of his which is in press at the time this paper is being written. He reports the findings of an experimental study made in an attempt to find a probable connection between the blood gland and the chromatophore activator. The results of a similar study on *Uca* have already been published (Carlson, 1935). According to Carlson, the middle third of the eye-stalk of *Uca* contains the active chromatophorotropic principle. Histological examination shows that the middle third of the stalk contains the well-developed blood gland, whereas the X-organ is either very small or completely absent. "In *Pagurus* . . . the blood gland . . . extends through both proximal and middle thirds of the eyestalk. The X-organ is situated in the proximal third, which thus contains part of the blood gland and the whole X-organ. Since the middle third of the eyestalk of *Pagurus,* that contains only part (the greater part, however) of the blood gland, concentrates the red and yellow pigment at least as strongly as the proximal third does, this fact, in connection with those mentioned above, speaks in favor of the view that the blood gland is the real source of the pigment concentrating substance in the decapod crustaceans" (Hanström, 1935). As Hanström recognizes, these facts do not exclude the X-organ from some part in the control of crustacean color changes. A more detailed knowledge of the rôles played by these two structures must await results from experimental removal or destruction of the endocrine tissues.

The second of these methods, used in this study, demonstrates fairly convincingly, that some endocrine organ within the eye-stalk is effective in causing two of the retinal pigments to migrate from a position that is characteristic for adaptation to darkness into a second position which is typical for an eye adapted to light.

It is interesting to note, in connection with this result, that a greater amount of migration of the distal pigment is obtained with the use of extracts prepared from eyes that were adapted to light, than with the use of extracts of an equal number of eyes which had been adapted to darkness. The explanation of this probably lies in the manner in which the eye-stalk hormone is synthesized and released by the animal. If the hormone is being formed continuously and stored until the time of release by light, acting as the stimulus, then there should be no difference in the potency of extracts prepared from light-adapted eyes and those

prepared from eyes adapted to darkness. If, on the other hand, no hormone is being built up in darkness, the response which was obtained from extracts of eyes adapted to the dark-room might be attributable to residual amounts of the effective hormone present in the gland through previous activity in response to light. This possibility merits cytological investigation.

In 1900, Gamble and Keeble reported a case of persistent rhythmic change in the chromatophores of *Hippolyte varians* even though the conditions of light remained constant. Slome and Hogben (1929) found a similar case of diurnal rhythm persisting in the South African frog, *Xenopus lævis,* after the removal of the normal stimuli. This animal, while kept in constant darkness, shows the melanophores more contracted at midnight than at midday.

Welsh (1930*b*) was the first to report a similar condition for the retinal pigment of crustaceans. Although *Macrobrachium olfersii* and *Macrobrachium acanthurus,* both nocturnal animals, were kept under constant illumination, the *distal pigment* continued to show a diurnal movement, migrating distally at 6:00 P.M. and remaining in this position until 5:00 A.M. the following morning, when it returned to the proximal position characteristic for light-adaptation. The proximal pigment did not exhibit such persisting rhythm.

Welsh's observations were soon followed by a similar one reported by Bennitt (1932*b*), who found that a rhythm persisted in the *proximal pigment* of the crayfish, *Cambarus,* even though the animal was kept in constant darkness. Subsequent studies made by Welsh (1935) found this phenomenon occurring in a number of other crustaceans. In *Latreutes fucorum* and in *Leander tenuicornis* the rhythm occurs in the *reflecting pigment* (and to a slight extent, perhaps, in the distal pigment) which, during the night, remains above the basement membrane in spite of constant illumination. *Leander affinis* shows "a definite rhythm in the movements of the reflecting pigment and a partial migration of the distal pigment" occurring "daily whether the animals are kept in constant light or darkness" (Welsh). *Penæopsis goodei,* a distinctly nocturnal form, possesses a persistent rhythm of the *proximal pigment,* being similar to *Cambarus* in this respect.

The physiology of these activities in crustacean retinal pigment is as yet little understood. Welsh concluded from his experiments with *Macrobrachium* that the normal migrations of the distal pigment cells as well as their periodic movements under constant conditions of light were controlled directly by the blood and indirectly by the nervous system. The mechanism underlying this phenomenon of persisting rhythm has been too little studied to warrant any final conclusion. The fact that a

persistent rhythm may exist for one kind of pigment in one species, and for a second or third kind of pigment in another species, that it may persist in constant darkness for one crustacean and in constant light for another, all add to the complexity of the situation. Are we to attribute these different rhythms to several different hormones, or is there only one hormone to which these various pigment cells respond differentially because, let us say, of threshold differences? In *Macrobrachium,* is the rhythm due to liberation of a hormone, or is it due to cessation of hormone secretion? Is the nervous system directly involved in rhythms of the proximal pigment? If endocrine glands are involved, are they in the position of intermediate agents, secretion being started or stopped by a rhythm in the nervous system, or do the glands themselves function in a rhythmical fashion? All of these possibilities must be considered and tested experimentally before we can proceed to an understanding of this intensely interesting phenomenon.

Summary

1. Specimens of *Palæmonetes vulgaris* which had been exposed to light so that their retinal pigments were in positions characteristic for that state were injected with crustacean eye-stalk extract, in one series prepared from animals that had been in the dark-room overnight, and in a second series prepared from specimens that had been kept on a black background. There was no significant change in the position of the distal retinal pigment cells in either series.

2. When stalk extracts were prepared from light-adapted animals and were injected, in the dark, into *Palæmonetes* in which the retinal pigments were adapted to darkness, a proximal migration of the distal retinal pigment occurred towards the position found typically in the eyes of specimens adapted to light.

3. If the rate of distal pigment migration following experimental injection is plotted, a sigmoid curve is obtained similar to that found by Welsh for the normal rate of migration when a single individual adapts to light.

4. Histological study of the eyes of experimentally injected *Palæmonetes* shows that the reflecting pigment as well as the distal pigment has migrated into a position typical for a light-adapted eye. The proximal retinal pigment apparently undergoes no change in position.

5. Stalk extracts which were prepared from animals that were adapted to darkness, and which were injected into dark-adapted *Palæmonetes,* in the dark-room, proved to be only half as potent as extracts prepared from the eyes of light-adapted shrimps.

6. Extracts of eye-stalks from *Cancer irroratus, Libinia dubia, Uca pugilator,* and *Carcinides mænas,* when injected into *Palæmonetes* which were adapted to darkness, also effected migrations of the distal and the reflecting retinal pigments. Stalk extracts from the eyes of *Callinectes sapidus,* in the concentrations used, had no effect on the retinal pigments of the test animals.

LITERATURE CITED

BENNITT, R., 1924. The migration of the retinal pigment in crustaceans. *Jour. Exper. Zoöl.,* **40:** 381.

BENNITT, R., 1932*a*. Physiological interrelationship in the eyes of decapod Crustacea. *Physiol. Zoöl.,* **5:** 49.

BENNITT, R., 1932*b*. Diurnal rhythm in the proximal pigment cells of the crayfish retina. *Physiol. Zoöl.,* **5:** 65.

BENNITT, R., AND A. D. MERRICK, 1932. Migration of the proximal retinal pigment in the crayfish in relation to oxygen deficiency. *Biol. Bull.,* **62:** 168.

BROWN, F. A., JR., 1933. The controlling mechanism of chromatophores in Palæmonetes. *Proc. Nat. Acad. Sci.,* **19:** 327.

BROWN, F. A., JR., 1935. Control of pigment migration within the chromatophores of Palæmonetes vulgaris. *Jour. Exper. Zoöl.,* **71:** 1.

CARLSON, S. PH., 1935. The color changes in Uca pugilator. *Proc. Nat. Acad. Sci.,* **21:** 549.

CASTLE, E. S., 1927. The interrelation of the eyes of Palæmonetes as concerns retinal pigment migration. *Proc. Nat. Acad. Sci.,* **13:** 637.

EWALD, A., AND C. FR. W. KRUKENBERG, 1882. Ueber die Verbreitung des Guanin, besonders über sein Vorkommen in der Haut von Amphibien, Reptilien, und von Petromyzon fluviatilis. *Untersuch. aus dem Physiol. Inst. der Univ. Heidelberg,* **4:** 253.

EWALD, A., AND C. FR. KRUKENBERG, 1883. Ueber Besonderheiten der Guaninablagerung bei Fischen. *Zeitschr. f. Biol.,* **19:** 154.

EXNER, S., 1891. Die Physiologie der facetterten Augen von Krebsen und Insecten. Leipsig u. Wien.

VON FRISCH, K., 1908. Studien über die Pigmentverschiebung im Facettenauge. *Biol. Zentralbl.,* **28:** 662, 698.

GAMBLE, F. W., AND F. W. KEEBLE, 1900. Hippolyte varians: a study in colour-change. *Quart. Jour. Micros. Sci.,* **43:** 589.

HANSTRÖM, B., 1931. Neue Untersuchungen über Sinnesorgane und Nervensystem der Crustaceen. I. *Zeitschr. Morph. u. Ökol. d. Tiere,* **23:** 80.

HANSTRÖM, B., 1934. Neue Untersuchungen über Sinnesorgane und Nervensystem der Crustaceen. III. *Zool. Jahrb. Anat.,* **58:** 101.

HANSTRÖM, B., 1935. Preliminary report on the probable connection between the blood gland and the chromatophore activator in decapod crustaceans. *Proc. Nat. Acad. Sci.* (in press).

HERRICK, F. H., 1891. Alpheus: A study in the development of Crustacea. *Mem. Nat. Acad. Sci.,* **5:** 370.

HOSOI, T., 1934. Chromatophore-activating substance in the shrimps. *Jour. Fac. Sci., Imp. Univ. Tokyo, Sect. IV,* **3:** 265.

KLEINHOLZ, L. H., 1934. Eye-stalk hormone and the movement of distal retinal pigment in Palæmonetes. *Proc. Nat. Acad. Sci.,* **20:** 659.

KOLLER, G., 1928. Versuche über die inkretorischen Vorgänge beim Garneelenfarbwechsel. *Zeitschr. vergl. Physiol.,* **8:** 601.

KOLLER, G., 1930. Weitere Untersuchungen über Farbwechsel und Farbwechselhormone bei Crangon vulgaris. *Zeitschr. vergl. Physiol.,* **12:** 632.

KOLLER, G., AND E. MEYER, 1930. Versuche über den Wirkungsbereich von Farbwechselhormonen. *Biol. Zentralbl.*, **50**: 759.

KROPP, B., 1932. The crustacean chromatophore activator and the gonads of the rat. *Proc. Nat. Acad. Sci.*, **18**: 690.

KROPP, B., AND E. B. PERKINS, 1933. The occurrence of the humoral chromatophore activator among marine crustaceans. *Biol. Bull.*, **64**: 28.

MILLOT, J., 1922. Signification biologique de l'argenture des poissons. *Bull. Soc. Zool. France*, **47**: 194.

MILLOT, J., 1923*a*. Le pigment purique chez les vertébrès inférieurs. *Bull. Biol. Fr. et Belg.*, **57**: 261.

MILLOT, J., 1923*b*. Influence de l'alimentation sur la pigmentation des vertébrès inférieurs. *Compt. Rend. Ass. Anat. Lyons.* (This reference I have been unable to obtain.)

MOSSLER, A., 1915. Die Pigmentwanderung im Auge von Pallaemon squilla. *Denkschr. kais. Akad. Wiss. Wien. Math.-naturwiss. Kl.*, **91**: 579.

NAVEZ, A. E., AND B. KROPP, 1934. The growth-promoting action of crustacean eye-stalk extract. *Biol. Bull.*, **67**: 250.

PARKER, G. H., 1891. The compound eyes in crustaceans. *Bull. Mus. Comp. Zoöl.*, **21**: 45.

PARKER, G. H., 1897. Photomechanical changes in the retinal pigment cells of Palæmonetes, and their relation to the central nervous system. *Bull. Mus. Comp. Zoöl.*, **30**: 273.

PARKER, G. H., 1932. The movements of the retinal pigment. *Ergebn. Biol.*, **9**: 239.

PERKINS, E. B., 1928. Color changes in crustaceans, especially in Palæmonetes. *Jour. Exper. Zoöl.*, **50**: 71.

PERKINS, E. B., AND B. KROPP, 1932. The crustacean eye hormone as a vertebrate melanophore activator. *Biol. Bull.*, **63**: 108.

POUCHET, G., 1876. Des changements de coloration sous l'influence des nerfs. *Jour. de l'Anat. et Physiol.*, **12**: 1, 113.

SJÖGREN, S., 1934. Die Blutdrüse und ihre Ausbildung bei den Decapoden. *Zool. Jahrb. Abt. Anat.*, **58**: 145.

SLOME, D., AND L. HOGBEN, 1929. The time factor in the chromatic responses of Xenopus lævis. *Trans. Roy. Soc. South Africa*, **17**: 141.

SMITH, D. C., 1930. The effects of temperature changes upon the chromatophores of crustaceans. *Biol. Bull.*, **58**: 193.

TROJAN, E., 1913. Das Auge von Palaemon squilla. *Denkschr. kais. Akad. Wiss. Wien. Math.-naturwiss. Kl.*, **88**: 291.

WELSH, J. H., 1930*a*. The mechanics of migration of the distal pigment cells in the eyes of Palæmonetes. *Jour. Exper. Zoöl.*, **56**: 459.

WELSH, J. H., 1930*b*. Diurnal rhythm of the distal pigment cells in the eyes of certain crustaceans. *Proc. Nat. Acad. Sci.*, **16**: 386.

WELSH, J. H., 1932. The nature and movement of the reflecting pigment in the eyes of crustaceans. *Jour. Exper. Zoöl.*, **62**: 173.

WELSH, J. H., 1935. Further evidence of a diurnal rhythm in the movement of pigment cells in eyes of crustaceans. *Biol. Bull.*, **68**: 247.

BINARY ANTERIOR OCELLI IN ANTS

WILLIAM MORTON WHEELER

(From the Biological Laboratories, Harvard University)

The opinion that the anterior of the three ocelli of insects really represents a pair of intimately fused ocelli is generally accepted by morphologists and is briefly expressed in several recent entomological handbooks. Thus Snodgrass (1935, p. 533) remarks that "there is evidence that the median ocellus has been formed by the union of two primitive frontal ocelli," and Imms (1934, p. 77) states more explicitly that "the median ocellus exhibits evidence of a paired origin since the root of the nerve supplying it is double, whereas the nerve-roots of the other ocelli are single. In some insects (e.g. Odonata, *Bombus*) the median ocellus exhibits a bilateral structure which is never found in the remaining ocelli." Imms' mention of the Odonata seems to refer to Hesse's observations (1901, p. 384) on *Agrion,* the median ocellus of which "has the same structure as the lateral ocelli, except that it is symmetrical. Whereas in other insects the duplicity of this eye is merely suggested by the double optic nerves, a bipartite condition is shown in *Agrion* also by the pushing in from the rostral side for some distance of a wedge of indifferent cells between the retinulæ."

Leydig seems to have been the first to detect the double innervation of the anterior ocellus. In his paper on the arthropod eye published in 1864 he says: "Concerning the optic nerves it may be said that the nerve of the median ocellus has *two roots,* each of which arises in one of the halves of the brain," and refers to the illustration (Fig. 4) of the brain of the *Formica rufa* queen in his atlas of comparative anatomy (1864*a*). This beautiful figure was reproduced by Sharp in his well-known treatise on insects in the Cambridge Natural History (1895, Fig. 65, p. 119). Among the later anatomists who have confirmed Leydig's observations on the double nerve supply of the anterior ocellus, Janet (1905) and Berlese (1909) may be mentioned.

Additional indications of the binary origin of the median ocellus are its conspicuously greater size as compared with each of the posterior ocelli in most worker ants which possess these organs and its sole persistence after the posterior ocelli have disappeared. If, however, there could be any doubt of the paired origin of the organ this has been removed by the study of its development. As early as 1887 Patten,

investigating the origin of the ocelli in *Vespa*, reached the following conclusions: "The *ocelli* of Vespa appear at the close of the larval period as *four* deep pits whose walls consist of a single layer of cells. The two anterior pits are situated close together, and soon fuse to form a single depression which develops into the anterior ocellus." . . . "The fact I desire to emphasize here, is that *the three ocelli arise from four single-layered pits, the median ocellus being formed from a coalescence of the two ventral ones*. The double nature of the median ocellus is also shown by the fact that, even in the latest stages, the root of the nerve is double, while that of the other two is single."

Apart from certain details such as the pit-like character of the ocellar primordia, Patten's observations have been confirmed by Redikorzew (1900), Zavřel (1902) and Caesar (1912). Although Redikorzew did not see the fusion of the two primordial thickenings in the honey bee, which he studied, he none the less observed the formation of the two nerves of the anterior ocellus and a very suggestive arrangement of the retinal pigment of all three ocelli. When first deposited in the retinulæ of the anterior ocellus the pigment has a remarkable bilateral distribution and that of each half is like the arrangement in each of the posterior ocelli. "From which it may be seen that each of the two halves of the median ocellus, so far as the pigment is concerned, has precisely the same structure as that of a whole lateral ocellus; in other words, it seems to be made up of two lateral ocelli."

Zavřel differs from Patten only in finding in *Vespa* that the four ocellar primordia are differentiated out of an even earlier, single "sense-plate." He gives some good illustrations of the double innervation of the anterior ocellus. Caesar has made the most thorough study to date of both the structure and development of the ocelli in ants. He describes the origin of these organs in the male of *Formica pratensis* as follows: "As in all the species of ants investigated the earliest primordia of the future ocelli make their appearance as a rule in the half-grown larvæ as four somewhat lens-shaped thickenings of the cerebral plate of the developing head-fold. Of these four primordia the two median, as shown most clearly in frontal sections, lie very close together and therefore constitute the double primordium of the future median ocellus, a proof that this ocellus has arisen phylogenetically by coalescence of two single ocelli." . . . "Concurrently with these developmental stages the lentigenous and retinogenous layers of the median ocellus fuse more and more intimately, but even in this stage a last indication of the former double origin of the whole primordium is retained in the two completely separate nerve-stands which connect it with the brain." The nearest analogue of the single anterior ocellus and its double nerve

supply among animals other than insects is the unpaired nasal sac of the cyclostome fishes with its paired olfactory nerves.

I have been led to consult the literature above cited by finding in some 15 among more than 4,000 gynandromorphs (ergatandromorphs) of a large neotropical ant, *Cephalotes atratus quadridens*, that the anterior ocellus is replaced by two smaller contiguous ocelli (Fig. 1).[1] Obviously, in these specimens the original paired primordia of the retinæ, corneagen cells and corneæ have differentiated in the adult without coalescing. Although it seemed probable that similar doubling of the anterior ocellus might occur occasionally among otherwise normal insects, I could recall no cases among the many thousands of Formicidæ

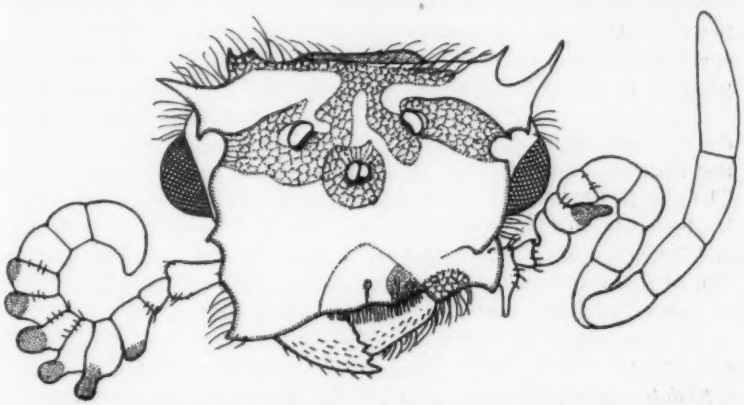


FIG. 1. Head of gynandromorph (ergatandromorph) of *Cephalotes atratus quadridens* Degeer, with binary anterior ocellus. One of several specimens. The large eyes, left mandible, thirteen-segmented left antenna, the stippled area and long hairs on the head and clypeus (except along its anterior border) and the papilla-like, stippled projections of the median joints of the right antenna are male characters.

and other aculeate Hymenoptera which I have examined during the past forty years. Moreover, my colleagues, Professor C. T. Brues, Dr. J. Bequaert, and Mr. Nathan Banks, who have a very wide acquaintance with many orders of insects, were equally certain of never having seen an example of double anterior ocellus and, like myself, could recall no reference to such an occurrence in the entomological

[1] This extraordinary number of gynandromorphs occurred in a single huge colony of *Cephalotes atratus quadridens* Degeer recently collected with great care by Dr. N. A. Weber in the island of Trinidad, B. W. I. A preliminary account of these anomalies was presented at the meeting of the British Association for the Advancement of Science in September, 1935. The final paper by Dr. Weber and myself has not yet been completed.

literature. At first sight, Comstock's general account of the ocelli in insects (1924) creates a suspicion that two ocelli may actually have been observed in some species. He says, p. 134: "When all [eyes] are present there are two compound eyes and, typically, two pairs of ocelli; but almost invariably the members of one pair of ocelli are united and form a single median ocellus. The median ocellus is wanting in many insects that possess the other two ocelli." On p. 135 he repeats this statement in a different form: "Of these [ocelli] there are typically two pairs; but usually when they are present there are only three of them, and in many cases only a single pair. When there are three ocelli, the double nature of the median ocellus is shown by the fact that the root of the nerve is double, while that of each of the other two is single." Since Comstock cites no cases of binary anterior ocellus, one suspects that the word "typically" in both these quotations is probably employed in the sense of "originally" or "phylogenetically."

After considerable search, however, I find that double anterior ocelli do occur in at least two species of myrmicine ants and I therefore believe that further investigation may reveal their presence in some other insects. The ant which exhibits this peculiarity most frequently is the huge-headed soldier, or worker maxima of the large neotropical leaf-cutting and fungus-growing *Atta cephalotes*. Linnaeus in his original description of this insect makes no mention of the ocelli, and Degeer (1773), who described it more carefully, expressly states that "the three smooth eyes are lacking." F. Smith in 1858 seems to have been the first to call attention to the large median ocellus in the worker maxima of *cephalotes* as distinguishing it from the same caste in *Atta sexdens*. According to Forel (1904) the maximæ of the former species "have three ocelli, of which the anterior is large, the two posterior small."

I have examined 300 maxima workers from series taken from some 25 colonies. Of these specimens, which comprise the typical *cephalotes* from the Guianas, Venezuela, and Trinidad, the subspecies *integrior* Forel (Brazil), the subspecies *opaca* Forel (Central America and northwestern South America) and *gorgo* subsp. nov. (Panama), more than 60 per cent have the anterior ocellus more or less distinctly divided.[2] In some of the series the percentage is even higher than 60, in others

FIG. 2. Front and ocelli of twelve specimens of the worker maxima of *Atta cephalotes*. *a*, subsp. *opaca* (British Honduras); *b* and *c*, subsp. *gorgo* subsp. nov. (Panama); *d*, *cephalotes*, typical (British Guiana); *e* and *f*, subsp. *gorgo* (Panama); *g*, *cephalotes*, typical (British Guiana); *h*, subsp. *opaca* (Ecuador); *i*, *cephalotes*, typical (Trinidad); *j*, subsp. *opaca* (Ecuador); *k*, subsp. *opaca* (Costa Rica); *l*, subsp. *opaca* (Ecuador).

[2] For a brief revision of these and other forms of *Atta* see my paper "Taxonomic Notes on the Ants of the genus Atta" (in preparation).

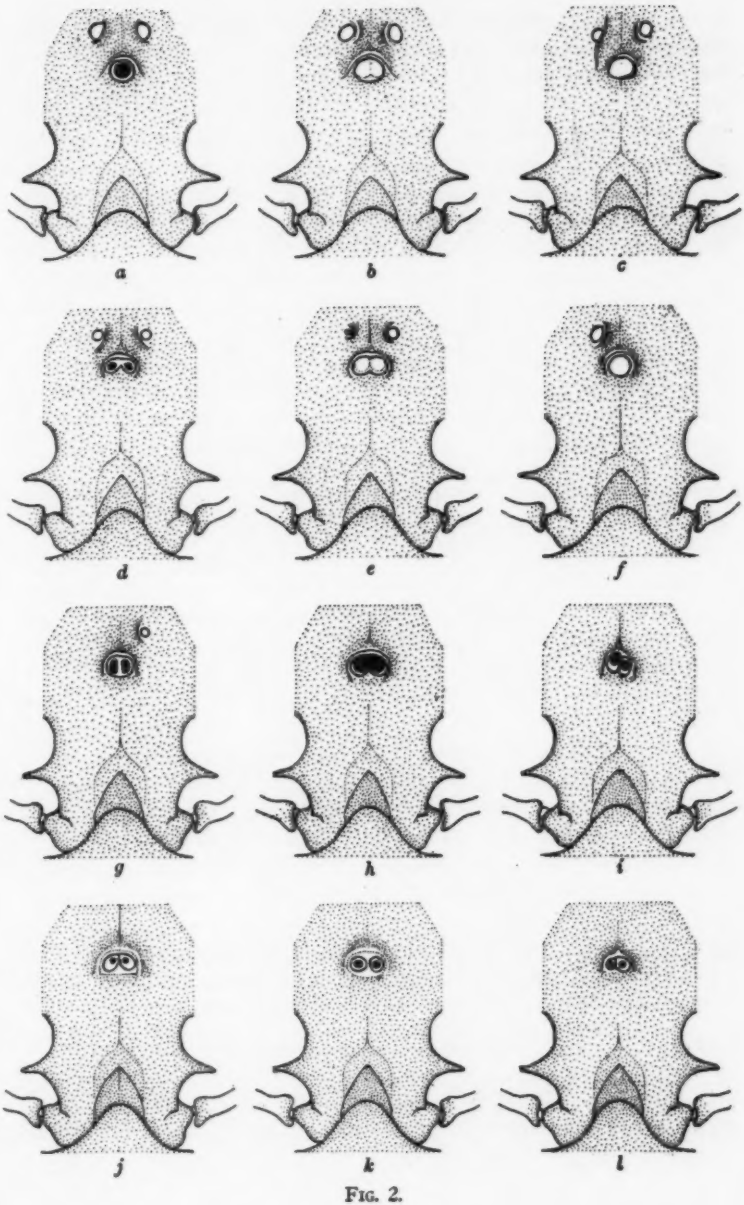


FIG. 2.

less. Thus in a series of 73 maximæ from a single flourishing and very pugnacious colony which Dr. William Beebe and I excavated at Kartabo, British Guiana, September 1, 1920, 93 per cent have a distinctly bipartite anterior ocellus. As shown in the 12 illustrations (Fig. 2), the ocelli are highly variable. Some specimens (*a–e*) possess all three

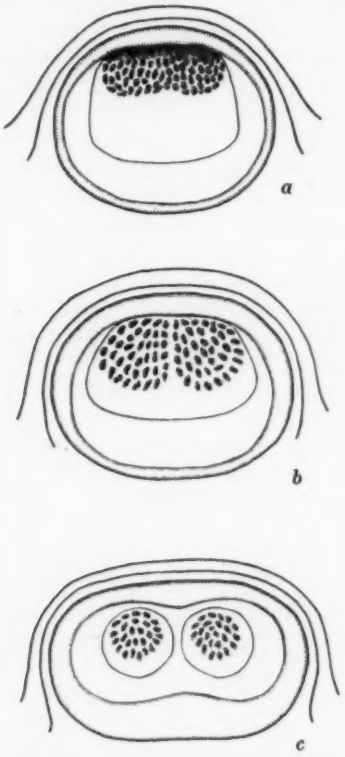


FIG. 3. Anterior ocelli of three maxima workers of *Atta cephalotes* from British Guiana seen from inside of head and showing distribution of retinal pigment under high magnification. *a*, retina with only slight and *b*, greater tendency to bipartition of retina. *c*, with two widely separated retinæ.

ocelli but one of the posterior is frequently smaller than the other (*c*, *e*) or as in *f* and *g* one of the pair has disappeared. In all cases each of posterior ocelli is decidedly smaller than the unpaired anterior ocellus. In alcoholic specimens this may have a single pigmented retina more or less clearly visible through the clear, undivided cornea (*a*), but all

stages may be observed from partially to completely divided corneas and/or retinæ (*b, d, e, g, i–l*). Occasionally one of the two members may be smaller (*l*) than the other or placed at a lower level on the front (*i*).

Portions of the front of maxima workers of *cephalotes* stained and mounted *in toto* and viewed from the inner surface of the integument under a high magnification showed little that could not be inferred from the outer surface, except the more definite arrangement of the pigment in the ommatidia. In the three preparations represented in Fig. 3 the amount of pigment in the anterior ocellus is approximately equal to that of both posterior ocelli, when these are present. In all my preparations the pigment of the anterior ocellus shows also that there are two retinæ which in some specimens are contiguous or even fused, in others separated by a very appreciable gap. The material, however, is so old and poorly preserved that other histological details cannot be discerned. The same statement applies to my sections of heads of pupal maxima workers. Hence before further investigation can be undertaken freshly fixed material will have to be obtained.

The other ant in which I have seen a double anterior ocellus is the Indomalayan *Pheidologeton diversus* Jerdon, a species which possesses a huge worker maxima like the same caste in *Atta cephalotes*. Usually only the large anterior ocellus is present, but occasional specimens also retain minute posterior ocelli. Unfortunately, my series of maximæ of this ant is rather small, but in one specimen belonging to the variety *laotina* Santschi from Saigon, the cornea of the anterior ocellus is distinctly bipartite.

The anterior ocellus in the largest workers of *Atta cephalotes* and *Pheidologeton diversus*, though large compared with either of the posterior ocelli, when they are present, is, nevertheless, a vestigial structure compared with its homologue in the cospecific male and female. When bipartite it obviously exhibits a retardation of development and persistence in a stage corresponding to the original paired hypodermal thickenings of the larva before or just after their union in the middorsal line, though the corneagen and retinogen cells are differentiated beyond this stage, as shown by the secretion of the cornea and the deposition of retinal pigment. I fail, however, to detect any differentiation of rhabdomes, but this is probably due to the poor preservation of the material.

Since, as a very general rule, the two primordia of the anterior ocellus in adult insects fuse to form a single organ, we are naturally led to inquire whether in certain species there may not be a coalescence of the primordia of both pairs of ocelli. There is, indeed, some evidence

of such an occurrence. It has long been known that what authors have interpreted as the posterior pair of ocelli in Plecoptera (Ephemerida) differ from those of all other insects in having an undeveloped cornea and beneath it a cellular lens. Now Seiler (1905) has shown that the anterior ocellus of *Ephemera vulgata* is really tripartite and is supplied by *three* nerves. This investigator is therefore inclined to believe that the so-called posterior ocelli of ephemerids are not homologous with the posterior ocelli of other insects and that the anterior ocellus is really a fusion of all three typical insect ocelli. The median of the three ocellar nerves, as shown in his figure, is twice as thick as either of the others and may therefore have arisen by coalescence of the pair belonging to the anterior ocellus. At any rate, renewed comparative study of the ocelli of ephemerids might yield results of considerable morphological interest, because Seiler's work clearly suggests that the ancestors of these insects were provided with three instead of two pairs of simple, in addition to the single pair of facetted eyes.

LITERATURE CITED

Berlese, A., 1909. Gli Insetti. Vol. 1, p. 572, Milan.

Caesar, J. C., 1912. Die Stirnaugen der Ameisen. *Zool. Jahrb. Abt. f. Anat.*, **35**: 15.

Comstock, J. H., 1924. An Introduction to Entomology. (First complete edition.) Ithaca.

Degeer, C., 1773. Mémoires pour servir à l'histoire des insectes. Vol. 3, p. 604.

Forel, A., 1904. Miscellanea Myrmécologiques. *Rev. Suisse Zool.*, **12**: 31.

Hesse, R., 1901. Untersuchungen über die Organe der Lichtempfindung bei niederen Thieren. VII. Von den Arthropoden-Augen. *Zeitschr. f. wiss. Zool.*, **20**: 347.

Imms, A. D., 1934. A General Textbook of Entomology. Third Edition. London, p. 77.

Janet, C., 1905. Anatomie de la tête du Lasius niger reine. Limoges.

Leydig, F., 1864. Das Auge der Gliederthiere. Tübingen.

Leydig, F., 1864*a*. Tafeln zur vergleichenden Anatomie. Tübingen.

Patten, W., 1887. Studies on the eyes of arthropods. I. Development of the eyes of Vespa with observations on the ocelli of some insects. *Jour. Morph.*, **1**: 193.

Redikorzew, W., 1900. Untersuchungen über den Bau der Ocellen der Insekten. *Zeitschr. f. wiss. Zool.*, **68**: 581.

Seiler, W., 1905. Beiträge zur Kenntnis der Ocellen der Ephemeriden. *Zool. Jahrb. Abt. f. Anat.*, **22**: 1.

Sharp, D., 1895. Insects, Part 1 in Cambridge Natural History. Macmillan Co.

Smith, F., 1858. Catalogue of Hymenopterous Insects in the Collection of the British Museum. Part VI. Formicidæ, p. 182.

Snodgrass, R. E., 1935. Principles of Insect Morphology. New York and London.

Zavřel, J., 1902. Untersuchungen über die Entwickelung der Stirnaugen (Stemmata) von Vespa. *Sitzungsber. k. böhm. Gesell. Wiss. math. naturw. Cl.*

THE BIOLOGY OF PSEUDOCALANUS MINUTUS IN THE GULF OF MAINE AND BAY OF FUNDY

CHARLES J. FISH

(From the Woods Hole Oceanographic Institution[1]*)*

Introduction

The present paper forms the second of a series on the biology of zoöplankton species in the Gulf of Maine and Bay of Fundy, with special reference to production and dispersal. As in the case of *Calanus finmarchicus* (Fish, 1936) there have been two objectives; first, a determination of breeding seasons, annual number of broods, and rate of growth; second, an evaluation of different spawning areas and dispersal of eggs and larvæ. For a description of the area covered, location of stations and methods, the reader is referred to the aforementioned report. The studies were carried on between July 28, 1931 and September 29, 1932 for the International Passamaquoddy Fisheries Commission.

Regional Distribution of the Adult Stock[2]

Pseudocalanus minutus was found by Bigelow (1926, pp. 276–277) to be nearly as universal as *Calanus* in the Gulf of Maine, distributed indifferently in the coastal zone, the deeper parts of the basin, and on offshore banks. It was somewhat more nearly universal close along shore but the numbers actually present averaged larger in the basin, entrant channels, and along the offshore slope. In 1931 and 1932 it was found generally distributed in both neritic and offshore waters.

Being more indifferent to neritic environmental factors than *Calanus* and with an apparently somewhat greater thermal range, *Pseudocalanus* occurs to some extent throughout the year in inshore waters north of Cape Cod. However, like other boreal arctic species, its numbers decline rapidly with rising summer temperatures in shallow areas, and offshore Bigelow (Ibid., p. 277) observed that there is a general tendency to leave the uppermost stratum in April and May.

In Passamaquoddy Bay in 1932 there was a decline in the relative percentage of *Pseudocalanus* from 84.7 per cent on June 20 to 2.8 per cent on July 30, with a corresponding reduction in the volume of plankton from 26.7 cc. to 1.9 cc. Later it rose again to 12.1 per cent ac-

[1] Contribution No. 94.

[2] "Adult stock" includes late copepodites (for the most part stage V) appearing in the coarse net hauls.

companied by an increase in volume to 13.3 cc. on September 23. McMurrich's unpublished data from St. Andrews in 1916 accord with these results.

In Frenchmans Bay, *Pseudocalanus* remains throughout the summer as one of the most important members of the zoöplankton community in the cold bottom water, but its center of abundance was found by day at a slightly higher and warmer level than that of *Calanus*.

South of Cape Cod *Pseudocalanus* largely disappears from the neritic zone during the summer months. In the Woods Hole region it remains abundant until May, declines rapidly in June after the water mass rises above 15° C., and disappears at about 20° C., reappearing usually in October (Fish, 1925, Fig. 45). *Calanus* occurs only during the coldest months in this area and is rarely ever numerous. Farther south in Chesapeake Bay *Pseudocalanus* is regularly found in the inner bay in the spring, but *Calanus* appears to be confined to the outer bay, where it is taken only in winter (Wilson, 1932, p. 13).

It would appear, therefore, that *Pseudocalanus* regularly outnumbers *Calanus* in observed neritic localities south of Cape Cod, and north of the Cape a similar condition occurs occasionally in some coastal areas during the early summer.

Production and Dispersal of Eggs and Larvæ

Spawning Areas

The distribution of eggs and early nauplii in 1931 and 1932 indicates that *P. minutus* spawns throughout the Gulf and Bay of Fundy. Even in neritic areas such as Passamaquoddy Bay where no evidence of *Calanus* propagation was found, 2,847 *Pseudocalanus* eggs per minute were taken on June 20, and 760 per cubic meter at the end of July in 1932.[3]

There is, however, considerable variation in the extent of propagation in different parts of the region. If the *Pseudocalanus* population in the inner Gulf and Bay originated largely in the western coastal area, as appears to be the case with *Calanus*, one would expect the quantitative distribution of larvæ of the two species to be very similar. The data obtained in 1932 indicate striking differences. Whereas the numbers of *C. finmarchicus* in all stages declined uniformly to the eastward (Fish, 1936, Fig. 11) and quantitative fluctuations in the western area were reflected throughout the region, in *P. minutus* the number of larvæ in the eastern basin and particularly in the outer part of the Bay

[3] Just outside of the bay in outer Quoddy waters (Sta. 5) *Calanus* eggs totalled 7,608 per minute and *Pseudocalanus* 37,018 on June 20, and *Calanus* 710 per cubic meter and *Pseudocalanus* 800 on July 30.

of Fundy was usually as large or even larger than west of Mt. Desert (Fig. 1).

This condition could have resulted either from intensive local spawning or large contributions from other areas. In regard to the first possibility, evidence of immigration in the horizontal distribution of all advanced larval stages of *P. minutus* indicates that production of this species in the eastern coastal region is on the whole probably no more successful than that of *C. finmarchicus* (Ibid., p. 140), *Sagitta elegans* (Huntsman and Reid, 1921, p. 110), and fishes having pelagic eggs (Bigelow, 1926, pp. 70–73; Huntsman, 1918, p. 65, 1922). Considering other possible sources of supply, as propagation in the western coastal area does not seem to have been sufficient to account for the in-

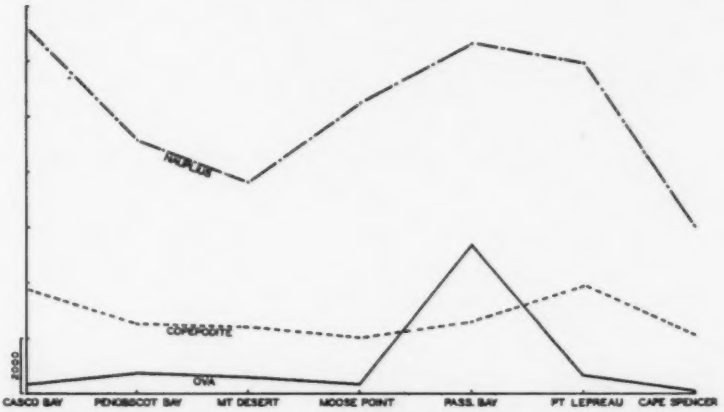


FIG. 1. Mean numbers of ova, nauplius and copepodite stages of *P. minutus* per minute of towing for the period April–July, 1932.

creasingly large numbers of *Pseudocalanus* occurring farther east (up to 215,698 late nauplii per minute in the Bay of Fundy in June) as the season advanced (Fig. 7*B*), it is probable that the principle spawning area of *P. minutus* is located in the outer Gulf where Bigelow (1926, p. 277) regularly found the species most abundant. The western coastal area appeared to be an important source for the first brood (Fig. 5*A*) in 1932, but thereafter declined until in mid-August there was little evidence of appreciable propagation except in a restricted locality represented by the innermost stations between Casco and Penobscot Bays (Figs. 8*A* and *B*).

Breeding Seasons

The breeding periods of *P. minutus* appear to be somewhat similar

to those of *C. finmarchicus*. As in the latter species, propagation begins earlier in the southern portion of its range and there appear to be a greater number of annual broods.

South of Cape Cod *Pseudocalanus* enters neritic waters in late October and breeding must soon follow because in 1922–23 early copepodite stages appeared at Woods Hole by the middle of December (Fish, 1925, p. 144). A maximum of copepodites in January was followed by a rise of adults in February. A second brood of less importance appeared in March and April. Some no doubt enter the region from the north where vernal augmentation takes place at this time, but in view of the abundance of adults at Woods Hole in February, it seems probable that the greater part of the March–April copepodites were of local origin. With the rise in temperature in May the numbers declined, and the species practically disappeared from inshore waters in June. Subsequent broods no doubt occur offshore where *Pseudocalanus* is found throughout the summer. The southern limit of successful propagation is not known but its presence regularly in Chesapeake Bay in winter and spring (Wilson, 1932) would suggest that there may be some successful spawning at that latitude during the cold months.

North of Cape Cod, Willey (1918, p. 187) reported females with eggs and attached spermatophores in August, and Bigelow (1926, p. 282) concluded that " the seasonal fluctuations in the numerical strength of the stock point to breeding as taking place most actively from June until September and to the entire gulf as its site."

Ruud's records (1929, pp. 67–68) indicate that the cycle in Norwegian waters is somewhat similar to that of *C. finmarchicus*. Spawning begins in March off Möre and probably earlier off the west coast of Norway. In 1926 there was a maximum of copepodite stages I–III on April 28–29. All copepodite stages had another maximum on July 20. This would suggest production in March, the young reaching maturity in May–June and the second generation attaining copepodite stages by July 20. Several observers have reported the presence of copepodite stages and mature adults in northern waters during the summer (Störmer, 1929, p. 31; With, 1915, p. 61; Bogorov, 1932).

In the Gulf of Maine and Bay of Fundy the seasonal distribution of young stages in 1931 and 1932 indicates that propagation begins in March and continues in a succession of generations throughout the region until September.

Regional variation.—Eggs of *P. minutus* appear at progressively later periods in the outer Gulf, western area, and Bay of Fundy, and, on the basis of this regional variation in the time of spawning, it is possible to distinguish three major breeding stocks.

These stocks are not permanently restricted to their respective localities, but merely represent delayed maturation of that portion of the winter population of *P. minutus* which happens to be located in the eastern part of the Gulf and Bay of Fundy during the spring, and earlier maturation in areas to the westward where the water mass responds more rapidly to vernal warming.[4] This is evident from the fact that the progeny of all stocks disperse and appear to become established over much if not all of the region, and also because within a region with a dominant drift it would be impossible to have permanently localized zoöplankton stocks without bottom stages. From the surface to the bottom the plankton population in any part of the open Gulf and Bay is in varying degrees a transient one. However, since the annual cycle in the three major areas starts at different times, with approximately the same interval of development (p. 200), one would expect the distinct breeding periods of the three stocks to be continued, as in *Calanus* (Fish, 1936, p. 130), in subsequent generations no matter where they might be dispersed. This is indicated in the present material.

In the following pages symbols will be used to designate the different stocks originating in the three general regions in the spring, and their respective progeny wherever distributed during the remainder of the season: *A* indicating the outer gulf stock, *B* that of the western coastal area, and *C*, that originating east of Mt. Desert in the inner Gulf and centered in the Bay of Fundy.

Annual Cycle in Different Breeding Stocks

Tracing the succession of broods in the three breeding stocks of *P. minutus* is more complicated than in *C. finmarchicus*. Unlike the latter species, where the bulk of the population of the Gulf apparently originates in one area (western) and can easily be traced, the broods of *P. minutus* are everywhere so largely depleted before copepodite stages are reached, that the particular stock which happens to be in early larval stages at the time dominates throughout the Gulf and Bay. Copepodite stages were almost always relatively sparse and in the present collections rarely formed appreciable maxima.

Since the succession of generations is most clearly defined in the stock maturing in the western coastal region in the spring, this stock (*B*) will be considered first. There appear to be at least three and possibly four breeding periods: March–April, May–June, July–August, and September. As seen in the quantitative distribution in successive

[4] It is probable that the transfer of adults from one area to another is relatively much slower than that of larvæ because, by descending during the daylight hours, they pass beyond the influence of the surface into a slower dominant drift.

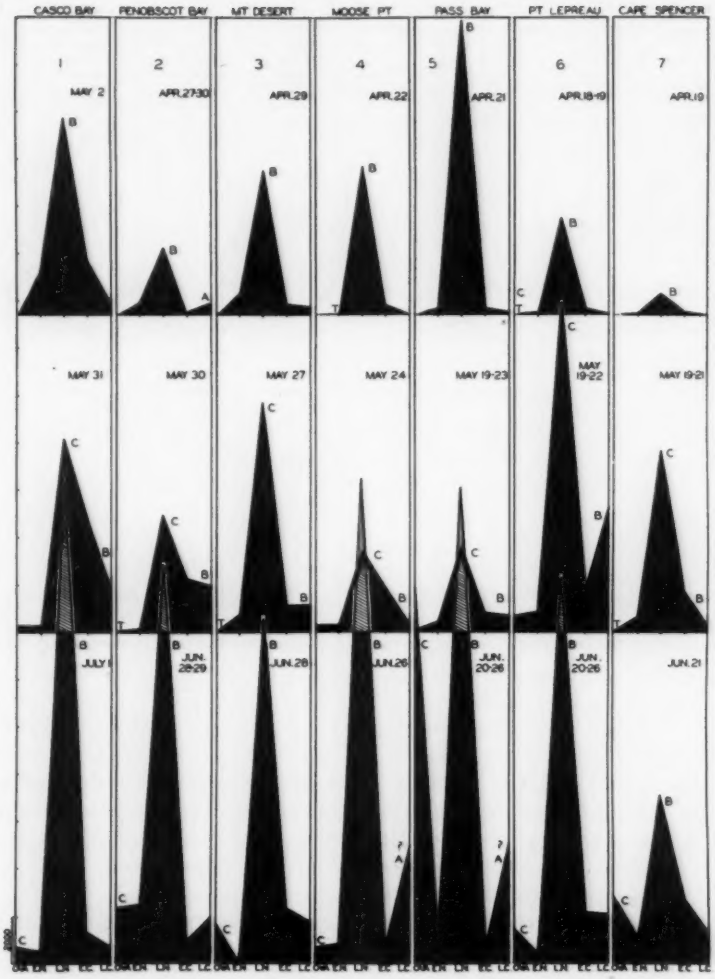


FIG. 2. Changes in the composition of the population of *Pseudocalanus minutus* (developmental stages) in seven sections between Casco Bay and Cape Spencer from April to June, 1932. Number per minute of towing. Stages recorded as: ova, early nauplii (I–III), late nauplii (IV–VI), early copepodites (I–III), and late copepodites (IV–V). *B* indicates stock originating in the western area in the spring, *C* indicates that of the eastern Gulf and Bay of Fundy, and *A*, the outer Gulf stock. *T*—trace.

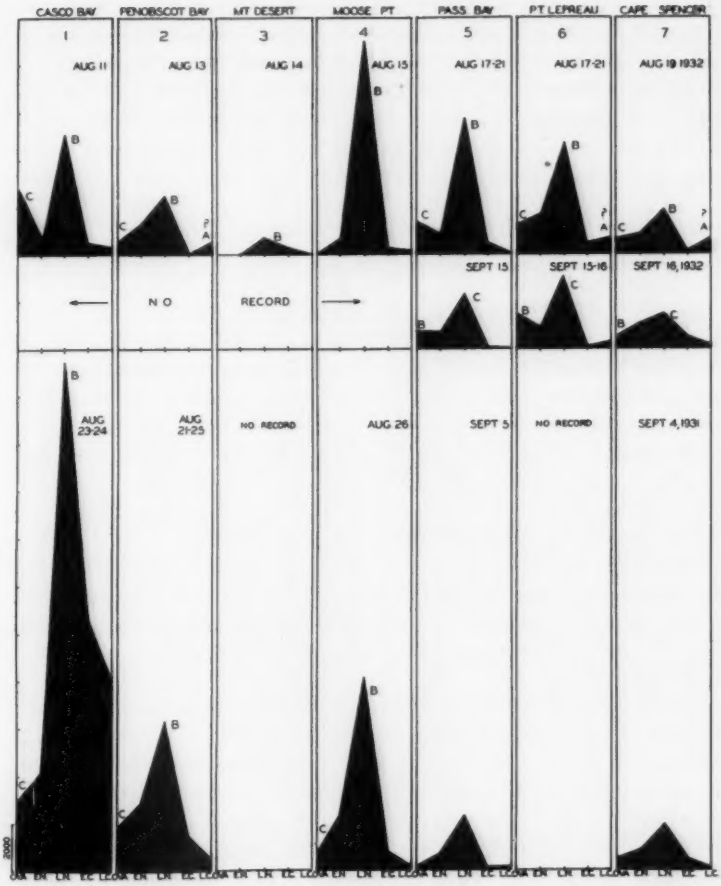


FIG. 3. Composition of the population of *Pseudocalanus minutus* (developmental stages) in August and September, 1931 and 1932. Number per cubic meter. Stages and symbols as in Fig. 2.

months, the first brood in advanced nauplius stages in late April had reached late copepodite stages on May 19–22 (Fig. 2, Section 6), and mainly completed the final moult by May 31 (Section 1). What is assumed to have been the second and largest western brood was represented by a maximum of late nauplius stages in the latter part of June. It would thus appear that the cycle during the early season is completed in about 60 days. The third brood, hatching on July 30 (Fig. 4), prob-

ably developed somewhat more rapidly with warmer summer temperatures and attained late nauplius stages by mid-August in the western area (Fig. 3). Even in the eastern Gulf at lower temperatures a large part of this stock passed from early to late nauplius stages between August 8 and 15, as indicated in Table I. Eggs entering the Bay of Fundy on

TABLE I

Stage	August 8, Sta. 15	August 15, Sta. 33
Egg	1,132	
Early nauplius	4,335	565
Late nauplius	5,842	12,060
Early copepodite	1,695	565
Late copepodite	377	377

September 15–16 would suggest a fourth generation but further data from the Gulf at this season are needed. It seems probable that a considerable percentage of the third crop remains in late copepodite stages to form the winter stock.

In stock C, originating in the eastern area, there is evidence of three breeding periods: April–May, June–July, and August. Local propagation was just beginning in the Bay of Fundy on April 19 (Fig. 2, Section 6) and a month later nauplius stages dominated. With a cycle of 60 days, eggs on June 20 (Fig. 4) would represent a second brood which by August 11–21 had matured and were spawning (Figs. 3 and 8*A–B*). A third brood is indicated by a maximum of late nauplius stages in the Bay of Fundy on September 15 in 1931 (Fig. 4), and September 15–16 in 1932 (Fig. 3).

Some less definite indications of a possible succession of generations in stock *A* after April are to be found in the data from the coastal region and the Bay.[5] In 1932, on June 20–26, when stock *B* was mainly in late nauplius stages, and the second brood of eggs of stock *C* had just appeared, a smaller maximum of late copepodite stages (Fig. 2, Sections 4 and 5) in the eastern basin may have represented a second brood of immigrants from offshore (Fig. 9*A*). With a developmental period of two months this would be expected. There is also an indication of a third crop of late copepodites on August 11–21, particularly in the western area (Fig. 3, Section 2) and the Bay of Fundy (Sections 6 and 7), but the numbers are too small to be of any real significance (Fig. 9*B*).

Although there was no detectable trace of stock *A* in May (Fig. 2), on the basis of the developmental period, the succession of subsequent maxima just described would appear reasonably indicative were it not

[5] In *Calanus*, either spawning in the outer gulf and western area coincides or the number of individuals of the offshore stock is insufficient to exert a detectable effect on the larval population of the inner gulf and bay.

for another complicating factor—the relatively small numbers of progeny attributable to this stock. There appears to be no logical reason for the depletion of one breeding stock of *Pseudocalanus* and the survival of another in the same locality. It is probable, therefore, that following the first generation of stock *A*, due to overlapping spawning periods, subsequent broods of this stock and stock *C* cannot with certainty be distinguished. At least a part of stock *A*, in copepodite stages

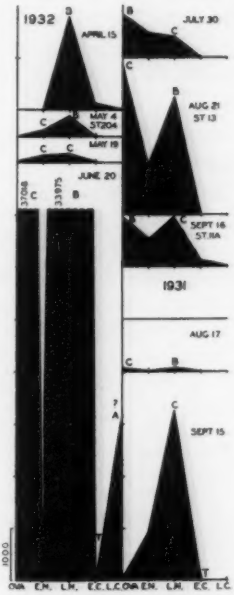


FIG. 4. Composition of the population of *P. minutus* in developmental stages at Station 5 (unless otherwise noted) in the New Brunswick area of the Bay of Fundy, showing the succession of generations of the (*B*) western, (*C*) eastern, and (*A*) outer Gulf stocks. *T* indicates trace. April–June: number per minute. July–September: number per cubic meter.

in the outer Gulf on April 11–12 (Fig. 5*B*), would be expected to have matured and started to spawn by the time eggs, forming the first brood of stock *C*, appeared in the Fundy region in the latter part of the month. In this case subsequent maxima, appearing in each instance one month after maxima of similar stages of stock *B*, would represent the combined stocks *A* and *C*, and the maxima of late copepodite stages [6] in late

[6] These copepodites, according to this interpretation, were probably derived from eggs appearing near the end of the previous spawning period and might be indicative of either one or both stocks.

June and August should be grouped with eggs appearing at the time. Further evidence in support of this contention will be presented later (pp. 209–211). In any event, with a similar rate of growth, it is reasonable to conclude that in stock *A* there are four broods, each of which appears about a month earlier than in stock *B* and two months or approximately one generation in advance of stock *C*.

Annual variation.—Observations at corresponding stations were made approximately two weeks later in 1931 than in 1932. As in the case of *Calanus* (Fish, 1936, p. 130) the season appeared about equally advanced in the western area, the slight increase in the percentage of copepodite stages off Casco Bay in 1931 (12 days later) being expected (Fig. 3).

In the central area east of Mt. Desert, and the Bay of Fundy, the fact that the relative numbers in comparable stages were approximately the same in the two years, would indicate that in 1931 the season was delayed about two weeks, a condition also according with *Calanus* (Ibid., p. 130).

TABLE II

Stage	Central Area		Bay of Fundy	
	1931 (Aug. 26)	1932 (Aug. 8–15)	1931 (Sept. 1–5)	1932 (Aug. 17–21)
Egg	750	566	225	1,041
Early nauplius	2,204	1,414	800	1,091
Late nauplius	8,117	8,810	2,937	4,112
Early copepodite	799	989	275	302
Late copepodite	188	330	112	442

Mortality in Developing Larvæ

The degree of mortality in developing larvæ of *P. minutus* can best be seen by comparing the relative abundance of different stages with those of *C. finmarchicus*. Of the two species *Pseudocalanus* must be by far the more prolific. The large number of spermatophores frequently found attached to individual females (Willey, 1919, p. 187, records as many as 24) suggests high egg production. The ovisac when attached contains usually from one to three eggs, but this may prove to be an adaptation for temporarily retaining the eggs to insure fertilization. Sars (1903, p. 21) observed that the sac is so fragile that it easily becomes detached at the slightest touch, and in examining Bigelow's collections (1926, p. 281) taken throughout the period of propagation in the Gulf of Maine, Wilson failed to find a single egg-bearing

female. Individuals with from one to two attached eggs were frequently taken in 1932, but to account for the numbers of free eggs of this species present at times in the region, several hundred must be produced by each individual. During the season of 1932, as shown in Table III,

TABLE III

1932	Eggs and young (all stages)		Relative percentage of adults	
Month	P. minutus	C. finmarchicus	P. minutus	C. finmarchicus
April	7,126	2,011	12.4	25.7
May	11,610	7,454	12.7	56.4
June	24,967	10,588	16.8	44.7
August	6,435	3,135	2.1	35.6
September	4,378	794	1.3	37.3

April–June: number per minute. August–September: number per cubic meter. Relative percentage of adults (including stage V) in the zoöplankton population.

although adults (including stage V) were outnumbered by *Calanus* in the total region by an average ratio of over 4 to 1, eggs and larvæ of *Pseudocalanus* dominated at all times with an average ratio of 2.5 to 1.

TABLE IV

Section	1932	P. minutus	C. finmarchicus
Casco Bay	April–June	17,248	13,137
	August	8,049	4,466
Penobscot Bay	April–June	12,442	9,490
	August	4,897	4,342
Mt. Desert	April–June	13,635	7,160
	August	956	1,860
Moose Peak	April–June	12,834	3,679
	August	10,181	5,184
Passamaquoddy Bay	April–June	20,494	4,749
	August–September	6,072	2,145
Pt. Lipreau	April–June	16,424	6,873
	August–September	7,164	1,516
Cape Spencer	April–June	8,893	1,665
	August–September	3,813	578

April–June: number per minute. August–September: number per cubic meter.

This is seen in Table IV to occur everywhere in the inner Gulf and Bay, the differences being greatest east of Mt. Desert.

Unlike *Calanus*, in which the depletion appears to be most extreme following the final moult (Nicholls, 1933, p. 95; Fish, 1936, p. 136), the decline in *Pseudocalanus* seems to be greatest in late nauplius stages.

There must also be a second critical period during maturation, which affects a far greater percentage of the stock than in *Calanus,* to establish the adult ratio because copepodite stages of *Pseudocalanus* exceeded those of Calanus during both the early and late summer seasons. (See Table V.)

Physical environmental factors appear to offer no logical cause for the depletion throughout the Gulf and Bay, and in the case of the late nauplii it seems to be attributable to destruction by enemies. Were there a lack of food, the relative decline in *Calanus* and *Pseudocalanus* larvæ would be expected to be similar.

Dispersal and Result on Regional Abundance of the Stock

The dispersal of eggs and larvæ from production centers and subsequent distribution of the adult stock are largely dependent on non-tidal circulation. The surface drift in the Gulf of Maine is counter-clock-

TABLE V

Total region 1932	April–June (Mean number per minute)		August–September (Mean number per cubic meter)	
Stage	P. minutus	C. finmarchicus	P. minutus	C. finmarchicus
Nauplius	10,568	2,160	4,476	638
Early copepodite	1,468	1,103	230	48
Late copepodite	1,079	514	292	10

wise, eddying around a central vortex. Eggs and larvæ produced in the western area would tend to be transported offshore and eastward around the outer margin of the Gulf. Outflow around the eastern end of Georges Bank as well as past Cape Cod would carry some of the larvæ, particularly those near the periphery of the drift, out of the region. However, an appreciable portion of the migrant stock or its progeny would be expected to remain within the Gulf completing the circuit across the entrance of the Bay of Fundy and westward along the coast of Maine, and some would continue eastward into the Bay along the Nova Scotian side.

One would therefore expect to find larvæ from the western area circling the outer Gulf and, with contributions from the latter area, supplementing the stock in the eastern basin and Bay of Fundy. Similarly, the greater number of eggs and larvæ originating in or entering the Fundy region would, if they survived, leave the Bay in the outflow which passes east and south of Grand Manan and tend to be dispersed westward in the inner gulf.

Dispersal in 1932.—Dispersal of individual stocks through successive generations has been traced by the monthly changes in the distribution of larvæ. Although stages ranging from ova to adults could usually be found almost everywhere in the region at any time, the maximum numbers, particularly of stock *B*, were in stages sufficiently separated from those of the other stocks to permit detection (Figs. 2 and 3).

Stock B.—Late nauplius stages, representing the first brood of that portion of the *P. minutus* population located in the western Gulf in the spring, were widely distributed throughout the region with local concentrations in the western and eastern basins by late April (Fig. 5*A*). A considerable part of the parent stock must have been transported well around the outer Gulf before spawning to account for such large numbers of nauplii in the Fundy region. There is a possibility, of course, that the larvæ might have been locally produced, but the sparseness of earlier stages east of Mt. Desert in the inner Gulf (Figs. 2 and 3) at this time and the appearance later of a distinct eastern brood (p. 200) warrants the conclusion that they were derived from parents transported from the western Gulf.

The concentration of late nauplii in a limited area off Halifax (Fig. 5*A*) indicates that the season, as in the case of *Calanus* (Fish, 1936), was as far advanced there as in the western coastal region. In the intervening region late nauplii were not found numerous until May, a condition somewhat comparable with the Bay of Fundy.

From April to May stock *B* declined greatly in numbers and by the time late copepodite stages were reached at the end of the latter month, quantities in excess of 1,000 per minute were found only in the inner Gulf west of Mt. Desert and over the central basin of the Bay of Fundy (Fig. 6*C*). The latter were no doubt migrants from the western or outer Gulf because copepodites were still very sparse as far west as Mt. Desert in the path of the outflow from the Bay, where one would have expected to find the stock present in the Fundy region in April if it had survived.

By the end of June important changes in the picture were noticeable. The second brood of stock *B* in late nauplius stages was, like the first brood in May, centered in the western coastal area and Bay of Fundy. In view of evidence of unsuccessful production in the latter area (p. 195), and the large number (21,410) of late nauplii in the entering drift on the Nova Scotian side, it seems probable that the April brood of stock *B* had become established in the general region of Georges Bank and the second brood of migrant larvæ from the western area were now being supplemented by increasingly large contributions from the outer Gulf. It was at this time (June 21) that the immense swarm mentioned on page 195 was encountered off St. John (Fig. 7*B*).

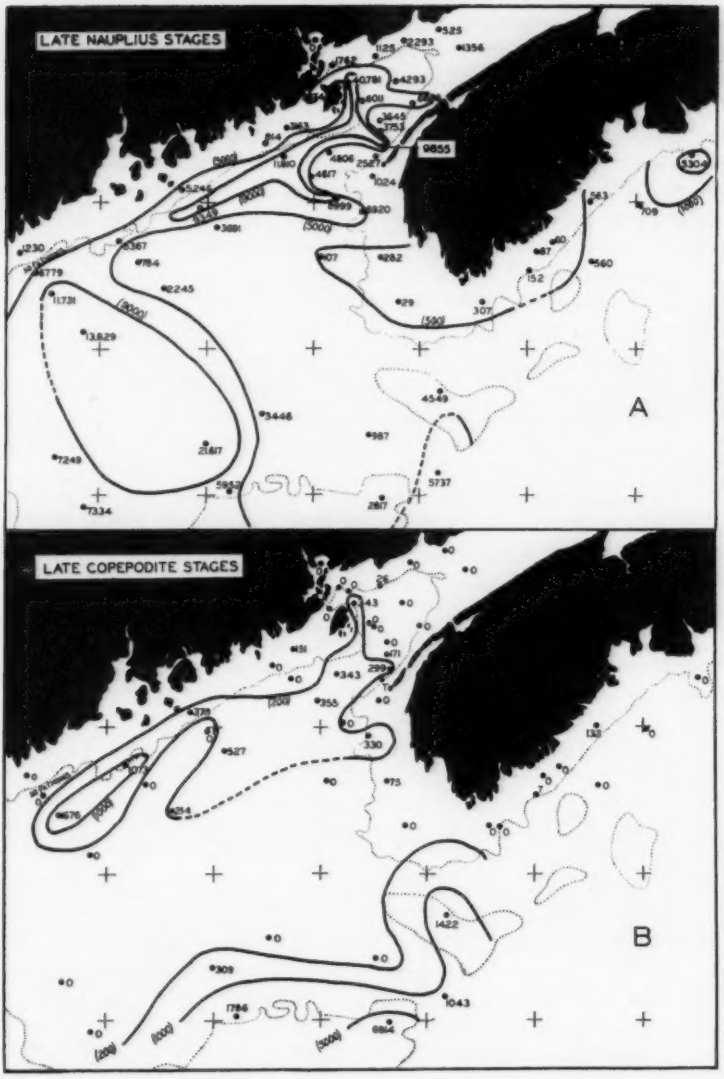


FIG. 5. Occurrence of late nauplius stages (*A*) of the western stock, and late copepodite stages (*B*) considered indicative of offshore propagation, in April, 1932. Number per minute of towing.

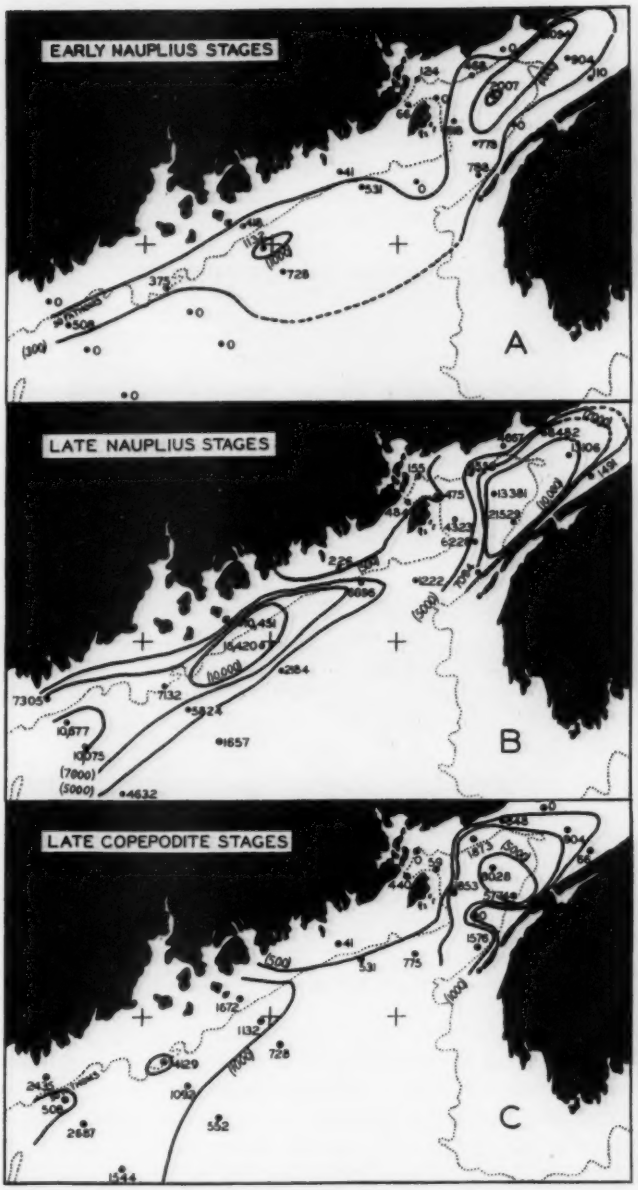


FIG. 6. Occurrence of nauplius stages (*A–B*) of the eastern stock, and late copepodite stages (*C*) of the western stock in May, 1932. Number per minute of towing.

There can be no doubt that by mid-August the larger part of the *P. minutus* population was originating in offshore waters. The third brood of stock *B*, by this time in late nauplius stages (Figs. 3 and 4), was, in the inner Gulf, definitely centered in the eastern basin and along the Nova Scotian side of the Bay of Fundy (Fig. 8*C*). Immigration of this population from the outer Gulf is indicated by the continued scarcity of larvæ in the New Brunswick area and the coastal zone of

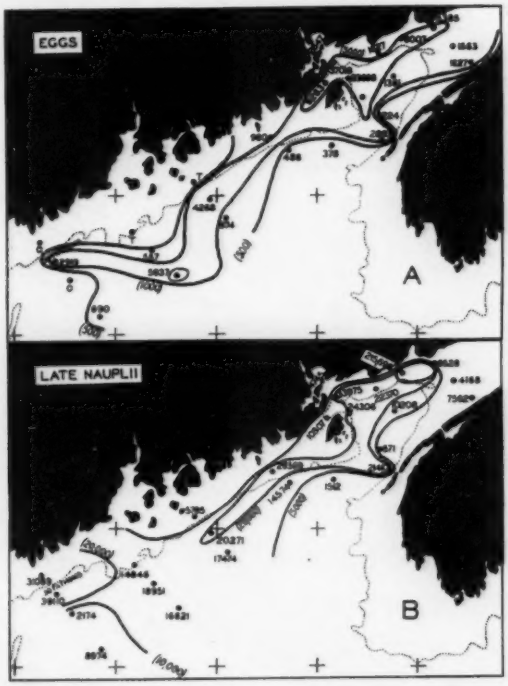


FIG. 7. Occurrence of eggs (*A*) of the second eastern brood and late nauplii (*B*) of the second western brood in June, 1932. Number per minute of towing.

eastern Maine beyond the immediate influence of the drift. The stock in the western area had declined until only at the innermost station off Casco Bay were more than 5,000 late nauplii per cubic meter taken.[7] If these conditions can be considered normal, then once the first brood of the stock originating in the western area in April becomes dispersed throughout the region, a considerable portion of the second brood, and

[7] Possibly the large population of predatory plankton animals in this region in summer depletes the *P. minutus* larval stock.

the greater part of the third, are derived from individuals establishing themselves in the general region of Georges Bank.

Autumn dispersal in the Gulf could not be traced as observations were restricted to the Bay of Fundy after August. By September both eggs (presumably the fourth brood of stock *B*) and larvæ of *P. minutus* had declined greatly in the Bay.

Stock C.—Originating from that portion of the *P. minutus* winter population maturing in the eastern Gulf and Bay of Fundy, stock *C* is believed to have been represented by small numbers of eggs at Station 37 in the Bay on April 20. Vernal augmentation must have just begun because eggs were not found elsewhere in April.

Differing from stock *B*, in which maturing adults of the parent stock had apparently become widely dispersed in the Gulf before spawning (Fig. 5*A*), propagation of the first brood of stock *C* seems to have been largely if not entirely restricted east of Mt. Desert. By May 18–24 late season eggs were not found outside of the Bay of Fundy;[8] early larvæ occurred at but two out of seven stations in the western area (Fig. 6*A*), and late nauplius stages (Fig. 6*B*), although more widely dispersed, were centered in the Bay of Fundy and along the course of the coastal drift from the eastward. It is probable that these late nauplii originated in the eastern basin and not in the Bay of Fundy because in the latter area the largest numbers were found in the course of the entering drift. Indicating local conditions beyond the immediate influence of this inflow, there was little evidence of survival in New Brunswick and eastern Maine coastal waters (Fig. 6*B*).

An analysis of the dispersal and resulting regional abundance of subsequent generations of this stock is rather complicated. Apparently the large numbers of late nauplii entering the western area in May (Fig. 6*B*) failed to establish themselves, for when this brood matured and were spawning in late June (Fig. 2) the numbers of eggs everywhere west of Mt. Desert were relatively small and at several of the stations none were found (Fig. 7*A*). This may have been due to destruction by enemies as previously suggested (p. 208) because the rich local population of plankton animals was at its maximum, and, as shown by conditions in August (Fig. 8), all of the breeding stocks of *P. minutus* were rapidly depleted in that area. On the other hand, corresponding to this decline in the western Gulf, there was a surprising increase of adults and eggs in the Bay of Fundy in late June. In the New Brunswick area adult *P. minutus* formed up to 83.2 per cent of the total zoöplankton population (Table VI, p. 213) and eggs up to 33,975 per

[8] Eggs, up to 1,405 per minute on May 19, were found only along the New Brunswick coast where the season appeared more retarded than elsewhere in the eastern region.

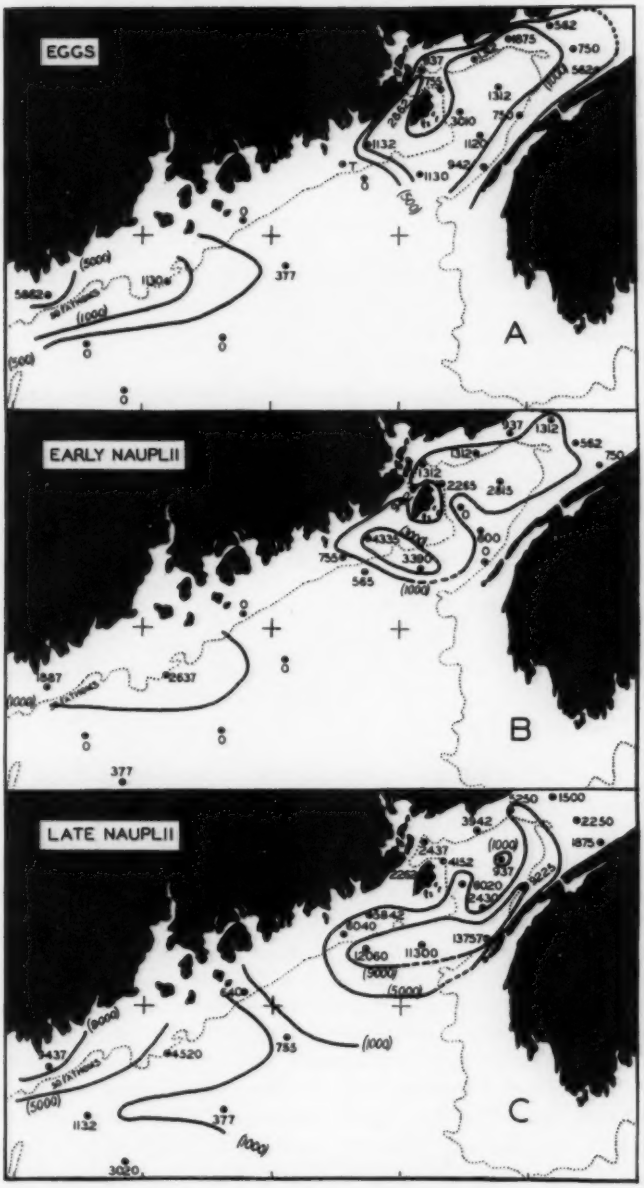


FIG. 8. Occurrence of eggs and early nauplii (*A–B*) of the third eastern brood and late nauplii (*C*) of the third western brood in August, 1932. Number per cubic meter.

cubic meter (Fig. 7*A*). The source of this population is somewhat uncertain, but, in view of the above-mentioned evidence of unsuccessful local production, it is believed that the adults consisted partly of members of the first brood of stock *C* from the eastern basin of the Gulf, and partly of the second brood of stock *A* from the outer Gulf (pp. 201–202). It is barely possible that propagation of this species is nowhere successful in the eastern Gulf and that all subsequent broods attributed to stock *C* were in reality migrants of stock *A*. This seems unlikely, however, since late nauplii off Mt. Desert in May (Fig. 6*B*) could hardly have been produced much farther away than the eastern basin.

By August further changes became evident. Eggs and early nauplii, presumably of the third brood of stock *C*, were concentrated in the Fundy region (Fig. 8*A* and *B*) and their scarcity or complete absence for some distance eastward from Mt. Desert would indicate that the depletion of the *P. minutus* population had now extended from the western Gulf to include the eastern basin. The similarity in the distribution of the two stocks, *B* (Fig. 8*C*) and *C* (Fig. 8*A* and *B*), in the Fundy region in August is most readily explainable on the basis that both were now centered in the same locality, and that the larvæ were derived from parents transported from the general vicinity of Georges Bank. Had observations been continued in September, it might have been possible to determine whether, with the decline of the predatory animal population, there occurred a progressive restocking to the westward in the inner Gulf by these migrants from offshore or their progeny. There is some indication that this may occur in Bigelow's (1926, p. 281) observation that there is "a greater absolute abundance over the area as a whole in late summer and autumn." Within the Bay of Fundy in mid-September, late nauplius stages of stock *C* (third brood) were most numerous (3,563 per cubic meter) in the entering drift from the Gulf and over the central basin.

Stock A.—Little can be said about the dispersal of this stock after April because of the probable confusion with stock *C* (p. 201). Originating offshore in March, by late April the larvæ, in late copepodite stages, were centered in the region of Georges and Browns Banks. Along the probable course of the drift smaller numbers had been dispersed to the outer part of the Bay of Fundy and along a tongue-like band penetrating the western area.

If copepodites in late June and August (p. 202) can be considered to consist in part of stock *A*, then this stock in the inner Gulf, at all times in the summer of 1932, remained restricted largely to the eastern basin and Bay of Fundy. One would have expected to find the progeny of the first brood (entering the western area in April) generally dis-

persed throughout the western Gulf, but the distribution of all copepodites in June (Fig. 9*A*) and again in mid-August (Fig. 9*B*) clearly indicated migration from the eastward.

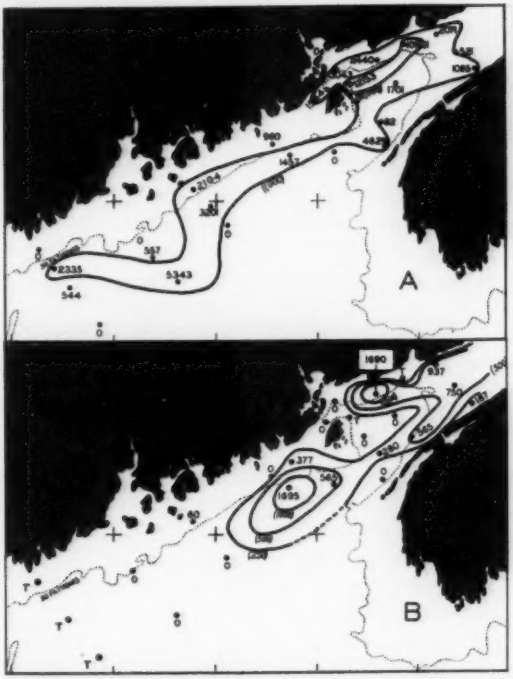


FIG. 9. Occurrence of late copepodite stages considered indicative of the outer Gulf stock in June (*A*) and August (*B*), 1932. June: number per minute of towing. August: number per cubic meter.

RELATIVE NUMERICAL STRENGTH OF PSEUDOCALANUS MINUTUS (ADULTS) IN THE ZOÖPLANKTON POPULATION

In the Gulf of Maine and Bay of Fundy *Pseudocalanus minutus* is second only in importance to *Calanus finmarchicus*. Offshore it is usually far outnumbered by the latter species, but in inshore waters such as the Quoddy region (Sta. 5, Table VI) and bays in the Gulf it is at times the dominant form. A similar condition is also found in the coastal region south of Cape Cod (Fish, 1925, p. 144, Fig. 45), and to the northward in the Gulf of St. Lawrence where Willey (1919, p. 187) found it constituting between 80 and 90 per cent of the copepod content between Prince Edward Island and the Magdalen Islands in May, 1915.

Combining the results of several years in the Gulf of Maine, Bigelow (1926, p. 282) found that *Pseudocalanus* averaged about 11 per cent of the copepod stock and as a rule was from one-tenth to one-fifth as abundant as *Calanus*. In 1932 during the period from April until September *Pseudocalanus* averaged 4.9 per cent of the total zoöplankton population of the Gulf, and *Calanus* 42.9 per cent, a ratio falling within Bigelow's range.

In the Bay of Fundy, based on monthly averages for three stations, the mean percentage of *Pseudocalanus* in meter net hauls for the year ending in August, 1932 was approximately 10, as shown in Table VI.

TABLE VI

1931–1932	*Pseudocalanus minutus*			
Month	Mean	Sta. 5	Sta. 6	Sta. 8A
September	2.3	3.7	2.8	0.5
October	1.5	1.5	—	—
November	1.9	2.1	3.3	0.3
December	3.1	4.5	2.3	2.5
January	8.2	8.2	—	—
March	12.0	6.6	10.9	18.5
April	5.8	5.5	5.8	6.0
May	22.0	53.2	8.0	4.8
June	43.4	83.2	45.2	1.7
July	3.0	3.0	—	—
August	5.3	15.2	0.8	0
Mean for Year	9.9	17.0	9.9	4.3

Along the New Brunswick shore (Station 5) it formed a much more important part of the plankton population than over the central basin or along the Nova Scotian side. Whereas *Calanus* increased in a seaward direction from the New Brunswick coast, the relative percentages of *Pseudocalanus* for the year decreased from 17 per cent in the Quoddy region to 4.3 per cent at the entrance to the Bay (Station 8*A*). This condition remained constant except for a short period following vernal augmentation when the percentage was greatest in the path of the drift from the Gulf. The highest value in the Bay (83.2 per cent) was found in the outer Quoddy region in June.

During the summer (April–September) the relative percentage of *Pseudocalanus* decreased westward from the Bay of Fundy, averaging 17.2 per cent in the Bay and as previously mentioned, 4.9 per cent in the Gulf, with minimum values in the western area.

Monthly averages indicated a definite seasonal variation in all areas covered in 1932 as the summer advanced. The highest value for the western area (10.6 per cent) was in April, for the central area (12.5 per cent) a month later in May, and for the New Brunswick area (59.2 per cent) in June. These results reflect the progressive response to vernal augmentation eastward along the coast. Indicating immigration from the western and outer Gulf, the Nova Scotian area yielded the highest percentages in March and April. Almost everywhere there was a continued decline after the first maximum, in spite of subsequent propagation.

The importance of *P. minutus* in the natural economy of the region is obviously greater than the relative numerical strength of the adult

TABLE VII

1932			Gulf of Maine			Bay of Fundy		
Cruise	Month	Total region	Total gulf	Western area	Central area	Total bay	New Brunswick area	Nova Scotian area
26	April	12.4	7.9	10.6	5.2	21.5	29.0	14.4
27	May	12.7	8.1	4.3	12.5	21.4	34.3	6.4
28	June	16.8	7.3	3.5	11.7	35.3	59.2	7.5
30	August	2.1	1.0	0.7	1.4	4.3	4.8	3.7
32	Sept.	1.3	0.1	0.3	T	3.6	2.1	5.1
Mean	April–Sept.	9.1	4.9	3.9	6.2	17.2	25.9	7.4

population would imply. Although, as Bigelow (1926, p. 282) has pointed out, the adult stock is still far behind *Calanus* because of its smaller size and fewer numbers, the enormous number of larvæ produced and subsequent rate of depletion indicate that its importance to those small predatory forms (including several of the more important species of Gulf fishes during their early existence) feeding on larval copepods must be considerably greater in some parts of the region than that of *Calanus*.

SUMMARY

1. *Pseudocalanus minutus* is generally distributed both in neritic and offshore waters. Having a somewhat greater thermal range it regularly outnumbers *Calanus* in neritic areas south of Cape Cod and on occasions in boreal waters also.

2. Propagation begins in March and continues in a succession of generations until September. It starts earlier in the southern part of the range where there are also a greater number of annual broods.

3. Eggs appearing at progressively later periods (approximately monthly intervals) in the outer Gulf, western area, and Bay of Fundy make possible a distinction of three major breeding stocks.

4. In the western stock there are at least three and possibly four breeding periods, March–April, May–June, July–August and September.

5. In the eastern stock there is evidence of three breeding periods, April–May, June–July, and August.

6. The outer Gulf stock is not clearly traceable due to probable overlapping of subsequent breeding periods with those of the eastern stock, but four periods are indicated.

7. There appears to be a developmental period of approximately two months throughout the region.

8. *Pseudocalanus* is very prolific and its early larvæ greatly outnumber those of *Calanus*. Mortality is relatively much higher in *Pseudocalanus*, the depletion being greatest in nauplius stages, although a second critical period during maturation is indicated.

9. There is no evidence of successful propagation in the Bay of Fundy, and the western area declines in importance after the first brood. Subsequent propagation of all breeding stocks appears to be centered in the outer Gulf.

10. The adult stock of *Pseudocalanus* is second only to that of *Calanus* in numerical importance, averaging 9.9 per cent of the total zoöplankton population in the Bay of Fundy during the year 1931–32 and 4.9 per cent of the summer population in the Gulf.

BIBLIOGRAPHY

Bigelow, Henry B., 1926. Plankton of the offshore waters of the Gulf of Maine. *Bull. U. S. Bur. Fish.*, **40**: 1.

Bigelow, Henry B., 1927. Physical oceanography of the Gulf of Maine. *Bull. U. S. Bur. Fish.*, **40**: 511.

Fish, Charles J., 1925. Seasonal distribution of the plankton of the Woods Hole region. *Bull. U. S. Bur. Fish.*, **41**: 91.

Fish, Charles J., 1936. The biology of Calanus finmarchicus in the Gulf of Maine and Bay of Fundy. *Biol. Bull.*, **70**: 118.

Huntsman, A. G., 1918. The effect of the tide on the distribution of the fishes of the Canadian Atlantic coast. *Trans. Roy. Soc. Canada*, **12**: 61.

Huntsman, A. G., 1922. The fishes of the Bay of Fundy. *Contr. Can. Biol.*, 1921 (1922), No. III, p. 49.

Huntsman, A. G., and Margaret Reid, 1921. The success of reproduction in Sagitta elegans in the Bay of Fundy and the Gulf of St. Lawrence. *Trans. Roy. Can. Inst.*, **13**: 99.

Nicholls, A. G., 1933. On the biology of Calanus finmarchicus. *Jour. Mar. Biol. Ass'n., N. S.*, **19**: 83.

PINHEY, K. F., 1927. Entomostraca of the Belle Isle Strait Expedition, 1923, with notes on other planktonic species: part II; and a record of other collections in the region. *Contr. Can. Biol., N. S.*, **3**: 331.

RUUD, J. T., 1929. On the biology of copepods off More, 1925–1927. *Rapports et Proces-Verbaux, Conseil perm. int. pour l'exploration de la Mer*, Vol. 56.

SARS, G. O., 1903. An account of the crustacea of Norway. Vol. 4, Copepoda Calanoida. Bergen.

STØRMER, L., 1929. Copepods from the "Michael Sars" Expedition, 1924. *Rapports et Proces-Verbaux, Conseil perm. int. pour l'exploration de la Mer*, Vol. 56.

WILLEY, A., 1919. Report on the copepoda obtained in the Gulf of St. Lawrence and adjacent waters, 1915. Can. Fish. Exped., 1914–15 (1918). Ottawa.

WILLEY, A., 1921. Arctic copepoda in Passamaquoddy Bay. *Proc. Am. Acad. Arts and Sci.*, **56**: 183.

WILSON, C. B., 1932. The copepod crustaceans of Chesapeake Bay. *Proc. U. S. Nat. Mus.*, Vol. 80, No. 2915.

WITH, C., 1915. Copepoda. I. Calanoida amphascandria. The Danish Ingolf-Expedition, Vol. 3, Part 4. Copenhague.

DIURNAL MOVEMENTS OF THE EYE PIGMENTS OF ANCHISTIOIDES

JOHN H. WELSH

(From the Bermuda Biological Station for Research, Inc., and the Biological Laboratories, Harvard University)

I

Physiological rhythms which have been established in organisms by regular environmental changes, such as the change in light intensity between day and night, occasionally persist when the external recurring stimuli are removed and the organism is kept under constant conditions. An example of such a phenomenon is seen in the movements of the eye pigments of certain crustaceans. The distal pigment cells in the eye of *Macrobrachium* continue to move back and forth along the ommatidia when animals are kept under constant illumination (Welsh, 1930*b*). The proximal pigment in the eye of *Cambarus* shows a partial migration, with periods corresponding with day and night, when animals are kept in constant darkness (Bennitt, 1932). Several species of Bermuda crustaceans show diurnal changes in one or more of the three sets of pigments of the eye when kept in light of a constant intensity or in constant darkness (Welsh, 1935).

When regularly recurring variations in external conditions are prevented, as they may readily be in the case of aquatic organisms, and the organism still responds as though in its normal environment, it is possible to account for the response only on the basis of an internal controlling mechanism. In the case of a diurnal rhythm the response is normally due to changing light intensity, temperature, humidity, or some other factor which varies with day and night. The response might be due entirely to the direct effect of one or more of these factors but if it persists under constant conditions it is evident that it is more complex than this and is probably effected by way of the nervous system or a combination of nervous and endocrine systems.

It would appear to be of rather general interest to have more detailed information on the nature of the controlling mechanism and the crustacean material is quite satisfactory for such an investigation. One fact that must be known in the case of the changes in the crustacean eye is how long the rhythm persists. The present paper is concerned primarily with this problem.

The experimental work was carried on in Bermuda and was in part made possible by a grant from the James F. Porter Fund. The author is indebted to Dr. J. F. G. Wheeler, Director of the Bermuda Biological Station, for his kind coöperation.

II

In 1933 two specimens of an unusual shrimp were taken in Bermuda by the author while collecting at night. In 1934 Dr. F. A. Brown obtained many more specimens which eventually proved to be *Anchistioides antiguensis* Schmidt, a form rarely taken in the Atlantic Ocean. Brown and Wheeler have been able to show (results not yet published) that this form is active only at night and can be collected in numbers only on the dark of the moon, thus showing a lunar periodicity. Because of its transparency, no pigment being present except in the eyes, it was selected for the present work. Preliminary observations on the eyes of animals kept in light of constant intensity or in constant darkness, except for short periods of examination, indicated slight diurnal changes in the distal pigment of the eye in the light, and complete normal daily movements in darkness. The appearance of such eyes when observed microscopically by means of transmitted light may be seen in Fig. 1, *A–D*. The condition seen in the eye of an animal kept in constant illumination and observed during the day will for convenience be called the "day-light" condition and may be seen in Fig. 1*A*. The same eye at night shows the distal boundary of the pigment nearer the periphery of the eye and this will be called the "night-light" condition (Fig. 1*B*). An animal removed from the dark during the daytime, and observed immediately, shows the distal pigment in the same position as is seen in the light during the day. This will be called a "day-dark" eye (Fig. 1*C*). If the same animal is returned to the dark and an examination made at night the entire eye is seen to be black except for a very thin border representing the cornea (Fig. 1*D*). This will be called the "night-dark" condition.

Observations of the intact eye show clearly the changes in position of the distal pigment cells and the changes which occur in the caps of reflecting pigment on the distal pigment cells, but changes in the main mass of reflecting pigment and in the proximal pigment can be seen only in sections of the eye.

The eye of *Anchistioides* as seen in sections is essentially like that of *Palæmonetes* which has been described by Parker (1897) and Welsh (1930*a*). The pigment changes are similar in most respects to those of *Palæmonetes* as described in these papers and may be compared with other crustaceans by reference to the review by Parker (1932).

Material for histological study was prepared in the following manner. A number of animals which had been caught the previous night were distributed among four containers. Two were illuminated with light from a 40W lamp at a distance of eighteen inches. Two were placed in a dark room. The period of adaptation began at 9:00 A.M. At 3:00 P.M. one lot was removed from the light and one from the dark and immediately fixed in hot water at 80° C. The heads were then cut off and placed in 70 per cent alcohol. At 11:00 P.M. the remaining two lots were treated in a similar manner. The eyes of a given lot of animals after sectioning showed the pigments in similar positions. Plate I, Fig. 3–6, show sketches of ommatidia based on camera lucida drawings. The situation is rather complex and each set of pigment must be followed through the series in order to make clear the changes which result from changes in light intensity and those which occur under constant conditions. The following summary supplements the figures and applies to the eye as a whole.

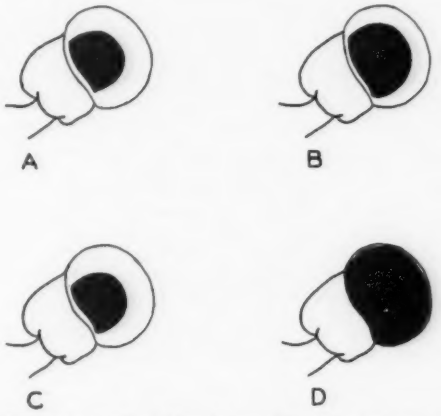


FIG. 1. *A*. Eye of an animal kept in constant illumination showing the position of the distal pigment cells during the day. A "day-light" eye.

B. Same eye as in *A* viewed at night. A "night-light" eye.

C. Eye of an animal kept in constant darkness except for short observational periods showing the position of the distal pigment cells during the day. A "day-dark" eye.

D. Same eye as in *C* viewed at night. A "night-dark" eye.

Day-light Eye. Fig. 3.

Distal Pigment Cells—In extreme proximal position resting on the retinular cells.

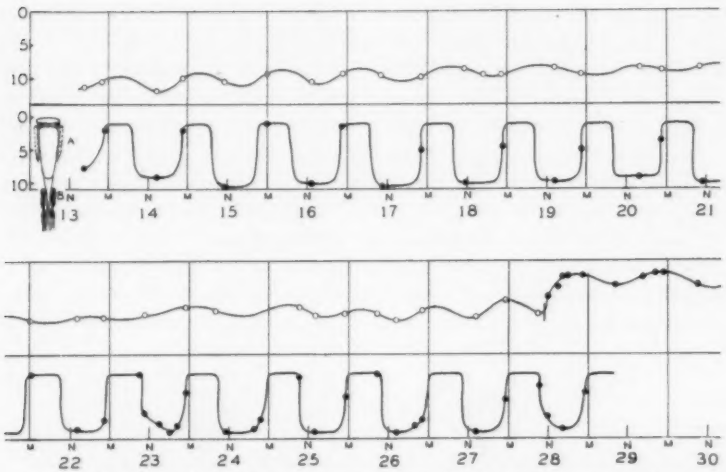


FIG. 2*A*. Records of the movements of the distal pigment cells of four animals kept in constant illumination (open circles), except for last two days, and of four animals kept in constant darkness (solid circles). Experiment started June 13 and ended on June 30. Noon and midnight are indicated by the letters *N* and *M*.

In the sketch of an ommatidium *A* is the extreme peripheral or night-position of the distal pigment cells, *B* the inner or day-position, in constant darkness.

The points as plotted are averages of the measurements of the distance from cornea to outer boundary of pigment. Each unit = 10 microns.

Time of sunset during course of the experiment about 7:35 P. M.

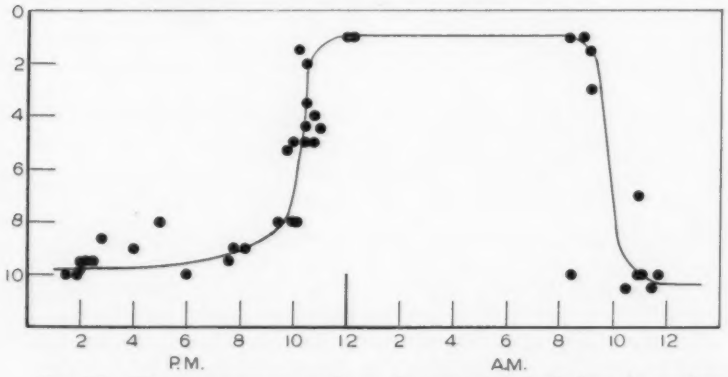


FIG. 2*B*. The measurements made on the eyes of animals kept in constant darkness and shown plotted over a period of fifteen days in Fig. 2*A* are here shown in one 24-hour period. Such a plot makes it possible to construct a curve representing the daily movements of the distal pigment cells. One of the coordinates shows the hours of the day beginning at noon, the other the distance of the distal pigment cells from the outer boundary of the cornea, each unit being equal to 10 microns.

Proximal Pigment—In retinular cells concentrated around rhabdomes. Some beneath the basement membrane.

Reflecting Pigment—Large masses resting on distal pigment cells, the remainder equally distributed above and below the basement membrane.

Night-light Eye. Fig. 4.

Distal Pigment Cells—Elongated but with proximal ends still in contact with retinular cells.

Proximal Pigment—Distributed as in day-light condition.

Reflecting Pigment—Most of this pigment which capped the distal pigment cells in the day-light conditions has moved proximally and fused with the mass below the basement membrane.

Day-dark Eye. Fig. 5.

Distal Pigment Cells—As in day-light condition.

Proximal Pigment—Above and below basement membrane. Very little in retinular cells.

Reflecting Pigment—Main mass around bases of retinular cells. Small caps on distal pigment cells and small masses below basement membrane.

Night-dark Eye. Fig. 6.

Distal Pigment Cells—In extreme distal position forming a collar around cones.

Proximal Pigment—Main mass below the basement membrane, none in distal part of retinular cells.

Reflecting Pigment—All is massed about the bases of the retinular cells where it forms a reflecting surface sending light back into the rhabdomes.

It is evident that the most striking changes in the eye which are independent of changes in the environment are seen when animals are kept in constant darkness. During the day the distal pigment cells are in the extreme inner position and during the night they move to the extreme outer position. In constant illumination, of the intensity used, the diurnal movement is not so striking as no change in position occurs, but only a change in length of the distal pigment cells.

It is not necessary to discuss further the changes in the reflecting pigment as they are clearly shown in the figures. The behavior of the reflecting pigment in certain other forms has been described elsewhere (Welsh, 1932). In this form the proximal pigment exhibits only slight

changes which are independent of light and in this respect is like *Macrobrachium*.

In the case of *Macrobrachium* it had already been found (Welsh, 1930*b*) that the distal pigment cells continued their characteristic movements when the animals were constantly illuminated, and it was possible to follow closely the time relations and determine that the outward movement began at the time of sunset and the inward movement at the time of sunrise. Similar observations were made on *Anchistioides* kept in the light and in the dark. On June 13 four animals in separate bowls of sea water were placed in the dark and four were placed in a situation where they received only light from a 40W lamp. During the course of the experiment they were not fed but the water was changed once or twice each day. They were usually examined once near midday and once at about 11:00 P.M. The distance from the cornea to the periphery of the central pigment mass was then measured by means of an ocular micrometer. In the case of the animals kept in the dark this meant a period of illumination of a few minutes twice in twenty-four hours. Measurements were made for sixteen days when the experiment in the dark was discontinued as two of the animals died on June 29. On June 28 the animals which had been in the light for fifteen days were transferred to the dark and measurements continued for two days and then stopped as our stay in Bermuda was at an end.

The results are shown graphically in Fig. 2*A*. The points as plotted are averages of the measurements on the four animals. Although there were individual variations they were not great and each animal responded essentially as the group as a whole. In the dark the movement of pigment was as extensive at the end of the experiment as at the beginning, and the only change was a slight lag which may be seen by following the positions of the pigment at 10:30 or 11:00 P.M. when measurements were usually made. If all of the measurements made on the eyes of

---

EXPLANATION OF PLATE I

Ommatidia (270 ×) showing details of pigment distribution.
Sketches are based on camera lucida drawings.

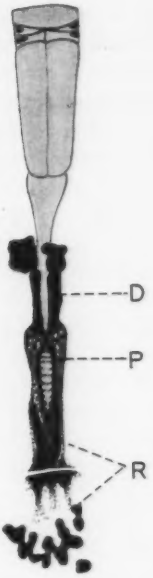
FIG. 3. Ommatidium from an eye of an animal constantly illuminated and killed and fixed during the day. "Day-light" condition.

FIG. 4. Ommatidium from an eye of an animal constantly illuminated and killed and fixed during the night. "Night-light" condition.

FIG. 5. Ommatidium from an eye of an animal kept in constant darkness and killed and fixed during the day. "Day-dark" condition.

FIG. 6. Ommatidium from an eye of an animal kept in constant darkness and killed and fixed during the night. "Night-dark" condition.

D = distal pigment; *P* = proximal pigment; *R* = reflecting pigment (the masses of reflecting pigment which rest on the distal pigment cells are not labelled).



3

4

5

6

PLATE I

animals kept in constant darkness are plotted in one 24-hour period, as seen in Fig. 2*B*, it is possible to draw a curve which represents quite accurately the movements of the distal pigment cells. The outward movement of the pigment which begins at about the time of sunset is slower than the inward movement occurring in the morning. This is in agreement with the results obtained in a study of the rate of migration of the distal pigment cells in the eye of *Palæmonetes* (Welsh, 1930*a*), where it was found that distal migration took approximately twice as long as proximal migration. The curve obtained here may now be applied to the data as shown in Fig. 2*A* and justifies the curves which are shown in this figure.

As has already been pointed out, the changes in position of the distal pigment cells, in constant illumination of the intensity used in this instance was not great; nevertheless it is possible to follow their rhythmical migration, which, at least for a few days, kept in phase with day and night. On June 17 and 18 an apparent lag is noticeable and on June 20, 21, and 22 the pigment cells seem to be out of phase with day and night. During this period the changes in position of the pigment cells were very slight and it is quite possible that this is an incorrect interpretation as later on June 25, 26, and 27 they are again in phase. A rhythm approximating a 24-hour one was present, however, and continued throughout the experiment. Some change resulted from the prolonged exposure to light for when the animals were placed in the dark the movement was not as extensive as in those animals which had been kept in constant darkness. It is probable that longer stay in the dark would have resulted eventually in a return to a more normal condition. It is also possible, although the experiment was not tried, that constant illumination of a higher intensity than that used would have completely suppressed the movements of the distal pigment cells.

III

The persistence of a rhythm in the movements of the eye pigments of *Anchistioides* for a period of more than two weeks indicates that it is no transitory phenomenon with which we are dealing. The mechanism which is responsible for the changes in the eye continues its cyclic activity long after all recurring external stimuli are removed. It is highly unlikely that the rhythm is a property of the pigment cells. That it is a property of one of the main coördinating systems, either nervous or endocrine, is more nearly possible. Some of the evidence to support this conclusion will be considered briefly. In the earlier work on *Macrobrachium* it was suggested that both the nervous and endocrine

systems were involved in the diurnal rhythm in the eye of this form. The results from the use of anæsthetics and the ligation of the eye-stalk led to this conclusion. The immediate control appeared to be hormonal yet somewhere in the chain the nervous system quite evidently played a part. At that time much less was known concerning the control of pigment migration in the crustacean eye than at the present time. Since then Kleinholz (1934, and in more recent unpublished work) has demonstrated, in a wide variety of decapod crustaceans, the part played by the so-called eye-stalk hormone in the activity of the eye pigments. It was reported (Welsh, 1935) that Kleinholz had found the eye-stalk extract controlled the inward movement of only the distal pigment cells. Since then the reflecting pigment has also been shown to be under the influence of material from the eye-stalk. No hormonal effect on the proximal pigment has yet been demonstrated. It is interesting to note that in *Anchistioides* the persisting movements are most extensive in the distal and reflecting pigments.

The work of Hanström (1931, 1934, 1935) and certain of his students (Sjögren, 1934; Carlson, 1935) has done much to further our knowledge of the sources of the eye-stalk hormone or hormones. These investigators have described two glands generally present in the crustacean eye-stalk which are quite certainly endocrine in nature. One they call the blood gland and the other the X-organ. Carlson has shown that the blood gland in *Uca* is the source of at least one of the pigment-concentrating substances affecting body chromatophores. The function of the X-organ still remains in doubt. These glands are supplied with nerves and their activities are doubtless controlled in part by impulses from the eyes arriving by way of the brain.

It was fortunately possible to have Dr. Hanström examine the eye-stalks of *Anchistioides* and he reported the presence of both the blood gland and the X-organ. The blood gland in this form is normally developed and is lying between the second and third optic ganglia on the dorsal side of the nerve mass. The X-organ is very well developed and is unusually far distal, almost touching the basal membrane of the eye. As in other decapods, its position is in the ventral part of the eye-stalk.

The hormone producing the movement, characteristic of the light, of the distal and reflecting pigments doubtless comes from one of these glands of the stalk. Normally we can assume that it is released into the blood stream under the stimulus of illumination, thence it is carried to the eye to produce so-called light adaptation of the eye. Under constant illumination in *Anchistioides* the production and secretion of the hormone continues and the pigment remains in a nearly constant light position depending on the intensity of illumination. In constant darkness,

however, the situation is not so obvious. Why should the hormone be released during daylight hours and not during the hours of darkness and why should this process continue for two weeks without becoming irregular or showing signs of disappearing? At present it is only possible to speculate. There may be a rhythmic secretory cycle in the gland which continues under constant conditions or the situation may be much more complex and the rhythm in the eye may only accompany a general rhythmic activity which results from a series of changes involving the nervous-endocrine systems. Experiments which are now being carried on may throw further light on this interesting phenomenon.

Summary

1. The distal pigment cells and the reflecting pigment in the eyes of *Anchistioides*, a crustacean, continue their characteristic movements under constant external conditions.

2. The movements of the distal pigment cells continued for fifteen days in animals kept in constant darkness and in constant illumination. Movements, normal in extent, occurred in constant darkness while in constant illumination the movements were greatly suppressed.

3. The persistence of this diurnal rhythm indicates that an internal cyclical mechanism is responsible, in part, for pigment movements in crustacean eyes.

4. The possibility of the blood gland or X-organ being concerned in the diurnal rhythm in retinal pigment movement is considered.

LITERATURE CITED

Bennitt, R., 1932. Diurnal rhythm in the proximal pigment cells of the crayfish retina. *Physiol. Zoöl.*, **5**: 65.

Carlson, S. P., 1935. The color changes in Uca pugilator. *Proc. Nat. Acad. Sci.*, **21**: 549.

Hanström, B., 1931. Neue Untersuchungen über Sinnesorgane und Nervensystem der Crustaceen. I. *Zeitschr. f. Morph. u. Okol.*, **23**: 80.

Hanström, B., 1934. Über das Organ X, eine inkretorische Gehirndrüse der Crustacean. *Psychiatrische en Neurologische Bladen*, **38**: 405.

Hanström, B., 1935. Preliminary report on the probable connection between the blood gland and the chromatophore activator in decapod crustaceans. *Proc. Nat. Acad. Sci.*, **21**: 584.

Kleinholz, L. H., 1934. Eye-stalk hormone and the movement of distal retinal pigment in Palæmonetes. *Proc. Nat. Acad. Sci.*, **20**: 659.

Parker, G. H., 1897. Photomechanical changes in the retinal pigment cells of Palæmonetes, and their relation to the central nervous system. *Bull. Mus. Comp. Zoöl.*, **30**: 275.

Parker, G. H., 1932. The movements of the retinal pigment. *Ergebnisse der Biologie*, **9**: 239.

Sjögren, S., 1934. Die Blutdrüse und ihre Ausbildung bei den Decapoden. *Zool. Jahrbücher, Abt. f. Anat.*, **58**: 145.

WELSH, J. H., 1930*a*. The mechanics of migration of the distal pigment cells in the eyes of Palæmonetes. *Jour. Exper. Zoöl.*, **56**: 459.

WELSH, J. H., 1930*b*. Diurnal rhythm of the distal pigment cells in the eyes of certain crustaceans. *Proc. Nat. Acad. Sci.*, **16**: 386.

WELSH, J. H., 1932. The nature and movement of the reflecting pigment in the eyes of crustaceans. *Jour. Exper. Zoöl.*, **62**: 173.

WELSH, J. H., 1935. Further evidence of a diurnal rhythm in the movement of pigment cells in eyes of crustaceans. *Biol. Bull.*, **68**: 247.

Out. - Maybe IN.

RENAL FUNCTION IN MARINE TELEOSTS

III. The Excretion of Urea [1]

ALLAN L. GRAFFLIN

(From the Department of Anatomy, Harvard Medical School)

The manner in which urea is handled by the kidney shows wide variations in the group of vertebrates. There is excellent evidence that urea is normally reabsorbed from the glomerular filtrate in considerable amounts by the renal tubules of the dog (Jolliffe, Shannon, and Smith, 1932; Shannon, 1935*a*; Van Slyke, Hiller, and Miller, 1935*a*, 1935*b*) and man (Shannon, 1935*b*; Shannon and Smith, 1935). Walker and Elsom (1931) have demonstrated that urea is freely filterable across the glomerular membrane of the frog, and conclude that "it is not necessary to assume tubular secretion of urea to account for the renal elimination of this substance in the frog." Marshall (1932), on the other hand, from xylose-urea comparisons, has concluded that urea may be secreted in large amounts by the renal tubules of the frog.[2]

For the marine teleosts the data are rather fragmentary. Marshall and Grafflin (1932), on the basis of glucose-urea urine/plasma ratios under phlorizin, were led to the conclusion that in the glomerular sculpin (*Myoxocephalus octodecimspinosus*) urea can be secreted by the renal tubule. Their experiments upon the aglomerular toadfish (*Opsanus tau*) indicated that urea is normally present in the urine in essentially the same concentration as exists in the plasma.[3] The striking observation was made that the sculpin kidney can be rendered functionally aglomerular by the administration of large doses of phlorizin; and under these conditions the urine/plasma urea ratio approaches the 1:1 value characteristic of the aglomerular toadfish. The aglomerular goosefish (*Lophius piscatorius*) is unique among the marine teleosts so far examined in that both plasma and urine consistently contain urea in only mini-

[1] The first two papers of this series appeared in the *Biological Bulletin*, Vol. 69, p. 391, and Vol. 70, p. 16.

[2] Even admitting that xylose gives values for the glomerular filtrate which are too low (see discussion below), it is hardly conceivable, in the light of our present knowledge, that Marshall's results could be explained on the basis of glomerular filtration alone.

[3] Bieter (1931) had previously demonstrated that urea exerts a marked diuretic effect upon the aglomerular kidney of this species.

mal amounts (see discussion below).[4] A few data upon urinary urea in *Gadus callarias* and *Spheroides maculatus* (both glomerular—Nash, 1931) have been reported by Smith (1929).

For the catfish, a fresh water teleost, Marshall (1932) reports finding urea concentration ratios lower than those of xylose, indicating a reabsorption of urea by the renal tubule.[5] In the marine elasmobranchs, which show a high degree of specialization in the tremendous urea content of their blood and tissues (Smith, 1932), the urea filtered at the glomerulus is almost completely reabsorbed by the renal tubules (Clarke and Smith, 1932). From the data available (Smith, 1931), this is likewise true in the case of the fresh water elasmobranchs, in which the blood urea level is much lower than in the marine forms.

The data reported in the present paper deal primarily with urine/plasma urea concentration ratios in the glomerular marine teleosts. Since this group of fishes normally shows a rather low rate of urine flow, but develops a marked diuresis under the usual experimental conditions (Grafflin, 1931, 1935; Pitts, 1934; Grafflin and Ennis, 1934; Clarke, 1934), the ability of the kidney to concentrate urea at widely varying urine flows has been investigated, particularly in the sculpin and to a lesser extent in the flounder. In addition, a few experiments are reported upon the behavior toward injected urea of the aglomerular kidney of the goosefish. This study was carried out at the Mt. Desert Island Biological Laboratory, Salsbury Cove, Maine.

Material and Methods

The animals used in this investigation were the sculpin (*Myoxocephalus octodecimspinosus*), flounder (*Pseudopleuronectes americanus*), daddy sculpin (*M. scorpius*), sea raven (*Hemitrypterus americanus*), wrymouth (*Cryptocanthodes maculatus*) and goosefish (*Lophius piscatorius*). The rate of urine flow was determined in all species but the goosefish in the following manner: the bladder was first emptied, with the use of a fine glass catheter, by massage of the overlying abdominal wall; the urinary papilla was then tied off with fine silk thread, and at the end of the collection period the urine was removed from the exposed bladder by syringe. The length of the collection period was variable: sculpin, 3 to 24 hours; flounder, 5 to 23 hours; daddy sculpin, 18 to 23 hours; sea raven, 18 hours; wrymouth, 7 hours. Many of the

[4] The data reported for the goosefish by Edwards and Condorelli (1928) cast doubt upon all of their urea analyses (see below), and their blood and urine figures for *Syngnathus*, *Hippocampus*, and *Muræna* will not be further discussed.

[5] Smith (1929) gives urinary urea data, without simultaneous blood analyses, for many species of fresh water teleosts.

fish were tied up immediately after catching, others only after a variable delay; and some were deliberately rendered diuretic by rough handling. All animals were unfed after catching, and the effect of starvation for a moderate length of time was studied especially upon six sculpins, which are marked with an asterisk in Table I. These fish were caught and handled throughout with every precaution, and were starved in laboratory aquaria for twelve days before the collection period was begun.

Sculpin, daddy sculpin, sea raven, and wrymouth were bled from the exposed heart by syringe, flounder from the severed caudal vessels. Bloods were drawn at the end of the collection period, rather than at the beginning and end or in the mid-period. The procedure used is perhaps to be preferred in view of Clarke's (1934) observation that a single bleeding in the course of an experiment may result in a marked interference with urine excretion. Marshall and Grafflin (1932) reported that plasma urea in the sculpin showed little variation in the course of twenty-four hours. However, a not inconsiderable variation was found in the toadfish in some experiments, and such a variation almost certainly occurred in many of the animals reported here. Since, from the toadfish results, the variation would presumably be in both directions, a group of animals would yield acceptable results, even though individual U/P ratios might not be accurate.

Goosefish were bled by syringe from the unexposed heart, and the bladder was emptied and urine collected by the use of a suitable catheter and massage of the overlying abdominal wall.

Urea was determined by the urease method in the Van Slyke apparatus (Van Slyke, 1927), with the use of 0.5 M veronal, rather than phosphate, as buffer because of the high magnesium content of the urine. All urea analyses with rare exceptions were done in duplicate, with the samples exposed to urease for three and six minutes. Heparin was used throughout as anticoagulant.

Results

Sculpin

The data on urea excretion in the sculpin are summarized in Table I, and demonstrate the following facts: (1) there is a considerable variation in the plasma urea level (5.3 to 18.7 mgm. per cent, with one value of 30.9 mgm. per cent); (2) the sculpin kidney is able to concentrate urea at all observed plasma urea levels and levels of urine flow, the degree of concentration being less in the higher range of flow (flow 3.7 to 15.2 cc., average U/P ratio 2.1; flow 17.4 to 52.0 cc., average U/P ratio 1.5); (3) starvation for twelve days (animals marked with an

asterisk in Table I) does not cause a detectable alteration in the plasma urea level (7.7 to 18.3 mgm. per cent) or in urea concentration by the kidney (flow 7.5 to 17.4 cc., average U/P ratio 1.9).[6]

The U/P ratios for urea become of particular interest when compared with Clarke's (1934) very careful study of the excretion of xylose, which he used, on the basis of previous work (Jolliffe, Shannon,

TABLE I

Renal excretion of urea in marine teleosts

SCULPIN				FLOUNDER			
Urea, mgm. per cent		U/P ratio	Urine flow	Urea, mgm. per cent		U/P ratio	Urine flow
Plasma	Urine			Plasma	Urine		
			cc. per kg. per 24 hrs.				*cc. per kg. per 24 hrs.*
14.6	24.6	1.6	3.7	13.3	23.3	1.8	8.3
30.9	62.2	2.0	7.5	11.2	10.9	1.0	10.3
13.3 *	24.4	1.8	7.5	9.3	11.6	1.2	10.4
12.7	32.8	2.6	7.8	14.4	23.0	1.6	13.0
10.5 *	14.4	1.4	7.8	8.5	14.0	1.6	13.2
10.8	19.5	1.8	8.3	18.4	25.9	1.4	18.9
11.0 *	27.6	2.5	9.3	11.3	15.6	1.4	26.4
7.7 *	17.2	2.2	9.5	12.1	13.2	1.1	26.7
4.6	9.1	2.0	10.2	10.1	12.8	1.2	37.0
9.5	18.2	1.9	10.5	12.3	11.7	1.0	43.0
5.5	16.1	2.9	10.7	11.3	13.6	1.2	45.6
18.3 *	34.1	1.9	11.0				
14.1	34.7	2.5	11.2	DADDY SCULPIN			
13.1	24.4	1.9	11.9				
15.6	44.0	2.8	15.2	14.0	19.6	1.4	3.2
10.7 *	18.9	1.8	17.4	7.8	8.5	1.1	10.5
6.7	7.9	1.2	18.2	10.2	10.2	1.0	37.6
5.3	7.8	1.5	19.2				
16.6	23.8	1.4	20.3	SEA RAVEN			
12.7	18.5	1.5	22.3				
18.7	28.8	1.5	31.7	8.2	7.3	0.9	8.3
11.9	17.6	1.5	44.5				
13.9	21.3	1.5	45.9	WRYMOUTH			
13.1	19.0	1.5	51.9				
14.8	16.7	1.1	52.0	6.7	22.1	3.3	11.3

and Smith, 1932; Clarke and Smith, 1932), as a measure of glomerular filtrate. His conclusion was that in the sculpin " with progressive diuresis there is a tendency for the increased urine flow to be effected by increasing glomerular filtration with a constant reabsorption of water

[6] For a description of the structure of the sculpin nephron, see Defrise (1932) and Edwards (1935).

(xylose U/P = 4) until the urine flow exceeds 10 cc. per kilogram per day; further diuresis is effected by a further increase in glomerular filtrate accompanied by a fall in the U/P ratio toward 1.0." More recent work (Richards, Westfall, and Bott, 1934; Shannon, 1934, 1935*a*, 1935*b*; Shannon and Smith, 1935; Van Slyke, Hiller, and Miller, 1935*a*) has led to the conclusion that xylose is reabsorbed to a considerable extent by the renal tubules, so that Clarke's figures for the glomerular filtrate must be considered to be too low. It is obviously unjustifiable for the present to draw any rigid conclusions from a comparison of Clarke's xylose data and ours on urea. However, the suggestions obtained from such a comparison merit consideration; namely, (1) that in the sculpin glomerular filtration alone seems adequate to account for all of the urea excreted; and (2) (and this applies primarily to the lower, more normal range of urine flow) that urea may be reabsorbed by the renal tubules in considerable amounts. In the light of our present knowledge, Marshall and Grafflin's (1932) earlier interpretation that urea may normally be secreted by the sculpin tubule is considered to be untenable without further proof. In view of the fact that a retention of nitrogenous metabolic products has been postulated to be of marked significance in the broad problem of osmotic regulation in the marine teleosts, and to be a necessary condition for the formation of trimethylamine oxide (Hoppe-Seyler, 1930*a*, 1930*b*; for discussion see Grafflin and Gould, 1936), it is very desirable that a further study of the excretion of urea and trimethylamine oxide in this group of fishes should be carried out simultaneously with an accurate measurement of the glomerular filtrate. At the present time inulin seems to be the substance of choice for this purpose.[7]

Flounder

The data upon flounder (kidney glomerular—Nash, 1931) indicate the ability of the kidney to concentrate urea, and it is noteworthy that the highest U/P ratios fall in the lower range of urine flow. Since no observations upon the measurement of glomerular filtration in the flounder are available for comparison, the probable mechanism of urea excretion in this species will not be discussed at this time.

Sea Raven, Wrymouth, Daddy Sculpin

The figures on single specimens of sea raven and wrymouth (kidneys of both species glomerular—Nash, 1931) are submitted without

[7] For a discussion of the excretion of urea and ammonia by the gills of fishes, see Smith (1929).

further comment. In regard to the daddy sculpin, it is interesting that the kidney of this species shows marked evidences of glomerular degeneration, so that some specimens are functionally entirely aglomerular (Grafflin, 1933). The fishes reported were of approximately the same weight (550–660 grams), but unfortunately no check upon their glomerular status was obtained.

Goosefish

In sharp contrast to the findings of Denis (1912–13; 1913–14) and Edwards and Condorelli (1928), who reported the presence of moderate to large amounts of urea in the blood and urine of the goosefish, was the observation of Marshall and Grafflin (1928) that they were unable to find more than a trace of urea in either the blood or the urine of this fish: plasma (or serum) 0.3 to 0.7, urine 0.2 to 0.5 millimols per liter. This observation was confirmed for the urine by Grollman (1929), who in four composite urine samples found the urea nitrogen to vary from 0.4 to 1.5 mgm. per cent (total nitrogen 56 to 373 mgm. per cent); and by Smith (1929), who in eight specimens found urea nitrogen values varying from 0.5 to 2.1 mgm. per cent (total nitrogen 31 to 60 mgm. per cent). The urea analyses of Marshall and Grafflin and of Grollman were carried out by the urease method in the Van Slyke apparatus (Van Slyke, 1927), those of Smith by decomposition with urease after extraction of ammonia with permutit; and it seems entirely reasonable to ascribe the results of Denis and of Edwards and Condorelli to the non-specificity of the methods employed.[8]

The goosefish is of particular interest because it is the only marine teleost so far reported in which urea is consistently present in the blood and urine in such minimal amounts. The kidney of this species is composed of large numbers of aglomerular tubules, but contains a relatively small number of glomerular structures (Edwards, 1928; Marshall and Grafflin, 1928), which Grafflin (1929) has shown to be incapable of function in the adult. That the aglomerular condition *per se* bears no essential relation to the urea findings in goosefish is indicated by the results obtained upon the aglomerular toadfish (Marshall and Grafflin, 1932).

Plasma-urine comparisons in the toadfish furthermore indicated that urea is present in the urine in approximately the same concentration as exists in the plasma. This finding led to the present experiments, designed to study the behavior of the aglomerular kidney of the goosefish toward injected urea. The low plasma and urine urea levels before in-

[8] Denis (1912–13), method of Folin (1912); Denis (1913–14), presumably the same method for urine, and the method of Folin and Denis (1912) for blood; Edwards and Condorelli, method of Condorelli (1922).

jection confirm those previously reported by Marshall and Grafflin, Grollman and Smith. These experiments demonstrate the rather striking fact that, after injection, the urinary concentration of urea is consistently significantly higher than the plasma level. One experiment is given in detail in the protocol. In two similar experiments, at lower

Goosefish; weight 12 kg.

		Urea in mgm. per cent		U/P ratio
		Plasma	Urine	
9:00 A.M.	Blood 1; urine 1. 40 cc. 50% urea intramuscularly	0.2	0.3	
10:10	Bladder emptied			
10:44	Blood 2	71.0		
11:12	Urine 2		95.0	
3:00 P.M.	Bladder emptied			
3:25	Blood 3	114.0		
3:51	Urine 3, 9.0 cc.		145.0	1.27
4:15	Blood 4	113.5		
4:42	Urine 4, 7.0 cc.		143.0	1.26

plasma levels, the results were as follows: (*A*) plasma urea 24.7, urine urea 30.9 mgm. per cent, U/P ratio 1.25; (*B*) plasma urea 53.1, urine urea 63.0 mgm. per cent, U/P ratio 1.19. Whether the significantly higher concentration of urea in the urine represents a fundamental characteristic of the tubular membrane in this species cannot be stated positively at the present time. Although there is as yet no evidence for the reabsorption of water by the aglomerular tubule, the possibility must be considered; and there is furthermore the as yet untested possibility that water may be reabsorbed from the urine somewhere along the drainage system of the kidney (collecting ducts, ureter, bladder).

Summary

Data upon plasma and urinary urea at varying rates of urine flow are presented for the sculpin and flounder. In addition, a few figures are reported for the daddy sculpin, sea raven, and wrymouth. From a comparison of the present data with those of Clarke (1934), glomerular filtration alone seems adequate to account for all of the urea excreted by the sculpin. Furthermore, urea may apparently be reabsorbed by the renal tubules in considerable amounts, particularly in the lower, more normal range of urine flow. In the aglomerular goosefish, in the plasma and urine of which urea is normally present in only minimal amounts, injected urea appears in the urine in significantly higher concentration than is present in the plasma.

LITERATURE CITED

BIETER, R. N., 1931. *Jour. Pharm. and Exper. Therap.*, **43**: 399.
CLARKE, R. W., 1934. *Jour. Cell. and Comp. Physiol.*, **5**: 73.
CLARKE, R. W., AND H. W. SMITH, 1932. *Jour. Cell. and Comp. Physiol.*, **1**: 131.
CONDORELLI, L., 1922. *Il Policlinico, Sez. Prat.*, **29**: 1366.
DEFRISE, A., 1932. *Anat. Rec.*, **54**: 185.
DENIS, W., 1912–13. *Jour. Biol. Chem.*, **13**: 225.
DENIS, W., 1913–14. *Jour. Biol. Chem.*, **16**: 389.
EDWARDS, J. G., 1928. *Am. Jour. Anat.*, **42**: 75.
EDWARDS, J. G., 1935. *Anat. Rec.*, **63**: 263.
EDWARDS, J. G., AND L. CONDORELLI, 1928. *Am. Jour. Physiol.*, **86**: 383.
FOLIN, O., 1912. *Jour. Biol. Chem.*, **11**: 507.
FOLIN, O., AND W. DENIS, 1912. *Jour. Biol. Chem.*, **11**: 527.
GRAFFLIN, A. L., 1929. *Am. Jour. Anat.*, **44**: 441.
GRAFFLIN, A. L., 1931. *Am. Jour. Physiol.*, **97**: 602.
GRAFFLIN, A. L., 1933. *Anat. Rec.*, **57**: 59.
GRAFFLIN, A. L., 1935. *Biol. Bull.*, **69**: 391.
GRAFFLIN, A. L., AND D. ENNIS, 1934. *Jour. Cell. and Comp. Physiol.*, **4**: 283.
GRAFFLIN, A. L., AND R. G. GOULD, JR., 1936. *Biol. Bull.*, **70**: 16.
GROLLMAN, A., 1929. *Jour. Biol. Chem.*, **81**: 267.
HOPPE-SEYLER, F. A., 1930*a*. *Verh. d. phys.-med. Gesell. zu Würzburg*, **54**: 160.
HOPPE-SEYLER, F. A., 1930*b*. *Zeitschr. f. Biol.*, **90**: 433.
JOLLIFFE, N., J. A. SHANNON, AND H. W. SMITH, 1932. *Am. Jour. Physiol.*, **100**: 301.
MARSHALL, E. K., JR., 1932. *Jour. Cell. and Comp. Physiol.*, **2**: 349.
MARSHALL, E. K., JR., AND A. L. GRAFFLIN, 1928. *Bull. Johns Hopkins Hosp.*, **43**: 205.
MARSHALL, E. K., JR., AND A. L. GRAFFLIN, 1932. *Jour. Cell. and Comp. Physiol.*, **1**: 161.
NASH, J., 1931. *Am. Jour. Anat.*, **47**: 425.
PITTS, R. F., 1934. *Jour. Cell. and Comp. Physiol.*, **4**: 389.
RICHARDS, A. N., B. B. WESTFALL, AND P. A. BOTT, 1934. *Proc. Soc. Exper. Biol. and Med.*, **32**: 73.
SHANNON, J. A., 1934. *Jour. Cell. and Comp. Physiol.*, **5**: 301.
SHANNON, J. A., 1935*a*. *Am. Jour. Physiol.*, **112**: 405.
SHANNON, J. A., 1935*b*. *Jour. Clin. Invest.*, **14**: 403.
SHANNON, J. A., AND H. W. SMITH, 1935. *Jour. Clin. Invest.*, **14**: 393.
SMITH, H. W., 1929. *Jour. Biol. Chem.*, **81**: 727.
SMITH, H. W., 1931. *Am. Jour. Physiol.*, **98**: 279.
SMITH, H. W., 1932. *Quart. Rev. Biol.*, **7**: 1.
VAN SLYKE, D. D., 1927. *Jour. Biol. Chem.*, **73**: 695.
VAN SLYKE, D. D., A. HILLER, AND B. F. MILLER, 1935*a*. *Am. Jour. Physiol.*, **113**: 611.
VAN SLYKE, D. D., A. HILLER, AND B. F. MILLER, 1935*b*. *Am. Jour. Physiol.*, **113**: 629.
WALKER, A. M., AND K. A. ELSOM, 1931. *Jour. Biol. Chem.*, **91**: 593.

BIOLOGICAL OBSERVATIONS ON THE MARINE FUNGI OF WOODS HOLE WATERS

F. K. SPARROW, JR.

(From the Woods Hole Oceanographic Institution[1] and the Department of Evolution, Dartmouth College)

INTRODUCTION

While the rôle played by marine bacteria in the decomposition of organic complexes is appreciated by all interested in a study of the ocean, the activities of fungi in this respect are practically unknown. Indeed, it may be justly said that the very existence of fungi which complete their whole life history in the sea is not known to most students of marine biology.

Occasional references to these rare and peculiar organisms may be found in the European literature from the 1860's onward. In the accounts of the early students of the seaweeds certain structures generally supposed by them to be the reproductive organs of the algæ were described and figured, which in many cases have been found to be, in reality, extraneous, parasitic fungi. In fact, practically all of the few marine fungi thus far found have been described as parasites or saprophytes of various marine algæ.

Representatives of four groups of the fungi have been discovered in marine waters, namely, the Myxomycetes (Labyrinthuleæ), Phycomycetes, Ascomycetes, and Fungi Imperfecti. Of the alga-inhabiting species which are truly parasitic or hemi-parasitic, those of the Phycomycetes seem to predominate. Many of these are not only able to initiate the infection of a healthy host, but once the alga has been killed have the capacity to live saprophytically on the dead contents, often in company with bacteria and Protozoa which have entered as secondary agents of destruction.

Only two papers have thus far appeared which deal at all extensively with marine fungi (Petersen, 1905; Sparrow, 1934) and both of these are concerned with forms collected in northern Europe. Twenty-three different organisms are described in these papers, all but one of which (*Pythium marinum* Sparrow) are ordinarily included in the order Chytridiales of the Phycomycetes. In our own waters two undoubted Phycomycetes have thus far been reported from the Pacific coast

[1] Contribution No. 99.

(Zeller, 1918), while the Atlantic side has apparently yielded but a single one (Martin, 1922). Recently, Papenfuss (1935) has mentioned a chytridiaceous parasite of the brown alga *Ectocarpus siliculosus* Dillw. which he states attacked about 50 per cent of the unilocular sporangia, but he does not name the organism. This material was collected in the vicinity of Woods Hole, Mass.

The present investigation was initiated with two points in mind: first, to determine whether or not marine Phycomycetes were actually present in Atlantic coastal waters—a point not altogether evident from the extant literature—and secondly, to determine, if such were found, the nature and extent of their activities in the cycle of decomposition of marine organic complexes. This paper embodies the results of the work done mainly during the summer of 1934, and, to a lesser extent, a portion of the summer of 1935 on the first of these objectives and includes not only an enumeration of the fungi found in the vicinity of Woods Hole, Mass., but in addition, pertinent notes on their morphology, life history, and taxonomy.

Fungi Collected

1. *Labyrinthula sp.* Saprophytic in cells of *Rhizosolenia spp.*, bottom of Great Harbor, August 14, 1934.

In debris dredged from the bottom of Great Harbor during a heavy "flowering period" of diatoms, large numbers of *Rhizosolenia spp.* were found in various stages of decomposition. In these cells there were often found the motile stage of a *Labyrinthula* (Text Fig. 35), sometimes in company with a Protomyxa-like protozoan, or a small chytrid. The fusiform swarm cells of the *Labyrinthula,* which were 12 μ long by 5 μ in diameter, made their way in and upon the algal cells by gliding along their tenuous pseudopodia. The most anterior of these cells, which was not preceded by any protoplasmic attenuation, displayed remarkable powers of amœboid movement. No solid particles could be detected within the swarm cells.

The uncertain taxonomic status of the species of this peculiar group does not allow of any specific designation of the present form. Morphologically it does not seem very different from *Labyrinthula macrocystis* described from marine algæ by Cienkowsky (1867), from the common inhabitant of diseased *Zostera* (Renn, 1934), nor, indeed, from certain fresh-water forms.

Very frequently in the debris which collects in the bottom of aquaria containing marine algæ, species of *Labyrinthula* are observed, and it is probable that they occur much more frequently than records in the literature indicate—a fact also evident from Miss Jepps's (1931) paper.

No cases of actual parasitism of algæ by these forms were observed, and at Woods Hole, at least, the organism on *Rhizosolenium* appears to lead a saprophytic existence. Experiments to test the pathogenicity of various strains of *Labyrinthula* are greatly needed.

2. *?Labyrinthula Chattoni* Dangeard. Parasitic(?) in *Cladophora sp.*, vicinity of Woods Hole, throughout the summer.

Very commonly in material of *Cladophora* which has been maintained in the laboratory for some time there will be found occasional greyish cells within which are rosettes of highly refractive material, generally surrounding a central mass of compacted brown granules—the remains of the host chloroplasts. These rosettes are divided radially into a varying number of segments from which at maturity numerous very minute amœboid bodies emerge. According to Dangeard (1932), these are the motile spores of the fungus, and he has figured numbers of spindle-shaped bodies which unite to form a net-plasmodium. In the present material no fusiform shape was ever assumed by the spores, although the bodies from which they emerged—considered by Dangeard to be sori—resembled closely those of his species; neither was a net-plasmodium formed, and for these reasons the writer considers a further study of the Woods Hole fungus necessary before definitely naming it.

3. *Protomyxa*-like parasite of *Bryopsis*. Parasitic in *Bryopsis plumosa*, vicinity of Woods Hole, summer of 1934, June, 1935; New Bedford Harbor, Mass., July 24, 1934.

In the course of studies made on a fungous parasite of *Bryopsis* (*Sirolpidium*) there was frequently found an exceedingly virulent parasite which is undoubtedly a protozoan. However, since the animal occurred in such abundance and very often in competition with the fungus, both usually consuming the entire contents of infected cells, it would seem of interest to include it here.

Infected cells were readily noted by their blackened appearance, a pathological symptom also produced by the fungus. The method of entrance of the parasite and the nature of the unit accomplishing this were not determined. Once established, the organism gradually enveloped part or all of the algal content which soon became discolored within its body. The shape of the invader was variable but in general resembled a giant amœba from any part of the surface of which delicate, often strongly contractile threads emerged. Very marked movement of these threads could be detected and frequently broadly fusiform or spherical enlargements containing algal material could be seen moving along them

(Plate I, Fig. 6) in much the same "trolly-wire" fashion as that observed in *Labyrinthula*.

In exhausted algal cells the animal seemed to go into a resting stage and to surround itself with a slightly thickened, rigid pellicle. The engulfed host contents were by now nearly black, and it was these cylindrical bodies with rounded ends which were so evident macroscopically.

A similar organism has been found very frequently in decaying *Rhizosolenia spp.* dredged from the bottom of Great Harbor and in dead cells of *Ectocarpus* from Gay Head.

4. *Ectrogella perforans* H. E. Petersen. Parasitic in *Licmophora abbreviata*, summer of 1934, June, 1935; *Striatella unipunctata*, summer of 1934, June, 1935; vicinity of Woods Hole; New Bedford harbor, July 24, 1934; Gay Head, Martha's Vineyard, Mass., July 16, 1934.

This virulent parasite is the commonest marine fungus in the vicinity of Woods Hole and during the summer it is almost impossible to find stands of *Licmophora* and *Striatella* not heavily infected with it. It not only occurs in all the littoral regions in the immediate vicinity of Woods Hole but has also been found in equal abundance in New Bedford Harbor and Gay Head. It is probable that it will be found along our coasts wherever the host plants grow. During the month of July in particular the fungus may occur in epidemic proportions (Plate III, Fig. 2). As many as 88 per cent of the diatoms (*Licmophora*) in a single mount containing many hundreds have been observed to be infected.

Since the present form presents no significant differences in morphological features from that recently described by the writer (1934) from Danish material found in the littoral of the Kattegat, it will not be dealt with in detail here, save for certain hitherto unknown phases.

In both *Licmophora* and *Striatella* there was ordinarily present in one infected diatom only a single, spherical, highly refractive thallus, although occasionally 2–5 were observed. This varied from 25–35 μ in diameter and lay loosely in the center of the host cell.

A good opportunity was afforded in several instances to determine the method of entrance of the parasite into the host cell, a feature not previously observed. Heavily infected colonies of diatoms contained many cells, to the outer surfaces of which adhered numerous minute (3 μ in diameter) quiescent cystopores of the fungus. The small size of these bodies made observations difficult, but in several cases spores were found from each of which a tenuous, needle-like penetration tube had emerged and had pierced the host wall. It is probable that, like other parasitic chytrids, the contents of the spore then flowed through the tube

into the algal cell, leaving the wall of the cyst outside. Such empty cystospores were found, although the actual passage of material into the cell was not witnessed. Once inside, the refractive fungous protoplasm soon assumed a central position in the host cell, possibly attaching itself to the nucleus of the diatom. Growth into the mature, somewhat spherical sporangium then ensued. The star-like arrangement of the chloroplasts of the host was soon destroyed and, as the fungus developed, their individual structure was broken down and their normal color lost. At no time was any host material observed to be ingested directly nor did the fungus exhibit any signs of amœboid motion. As the *Ectrogella* increased in size, the disintegrating host material usually became collected around the periphery of the parasite, where it gradually became reduced to a small amount of granular, chestnut-brown residue. The nature of this unassimilated material is not known.

There then took place a transformation of the contents of the thallus into innumerable, minute zoöspores. These were discharged through 1–5 stout tubes into the water. A point of great morphological and taxonomic interest was determined from the spores of this material. Petersen (1905) states that in this species they are anteriorly uniciliate, the cilium being directed posteriorly when the spore is in motion. In the Danish material previously observed, the writer (1934) was unable to determine the ciliation, but at Woods Hole, the spores were found to be biciliate. This finding was confirmed in many instances. The body of the zoöspore is pyriform, somewhat curved, and at the narrower, forwardly directed end there is usually a refractive droplet. The two, perhaps anteriorly attached, cilia are directed oppositely when the spore is in motion. The relative lengths of these two structures could not be determined. Motion through the water was of an uneven, twisting character. The resemblance of these spores to those of *Sirolpidium* (Sparrow, 1934) is very marked.

From these observations, the parasite can now with real reason be placed in the genus *Ectrogella* as amended by Scherffel (1925).

At no time during the process of disintegration of the host cell could the presence of any other type of organism be detected. Bacteriological stains were employed to determine whether or not bacteria assisted in the process of disintegration, but none was ever observed. Indeed, the fungus had apparently so completely absorbed the available nutriment that even after infected cells had become broken or had fallen apart, bacteria and Protozoa were seldom seen feeding on the residual material.

It was very evident that not only could the fungus initiate the infection, but once inside, could bring about, unaided by other agencies, the almost complete disintegration of the contents. Further, the thallus,

derived from a single zoöspore, at maturity became transformed into a myriad of swimming spores, each of which was potentially able to infect another diatom. Hence, in a region with an abundance of favorable host material, the rapid and widespread dissemination of the parasite could be quickly accomplished.[2] Indeed, it is remarkable in view of the high percentage of infection observed, that the two species of diatoms were able to maintain themselves at all. It is hoped that a more comprehensive study of this disease can be made in the future.

5. *Eurychasmidium tumefaciens* (Magnus) n. gen. Parasitic in the nodal cells of *Ceramium diaphanum*, Sheep Pen Cove, Woods Hole, August 27, 1934, collected by C. Jao; Little Harbor, Woods Hole, August 29, 1934, collected by the author.

Eurychasmidium n. gen. Plasmodial thallus entirely intramatrical, one-celled, filling the distended host cell, forming at maturity many discharge tubes which pierce the host wall; zoöspores biciliate, encysting near the sporangium after discharge, ultimately emerging as motile zoöspores. Resting spores not observed.

Thallus plasmodialis, omnino intramatricalis, unicellulatus, cellulam matricis distentam implens, maturitate formans multa ostiolata perforantia parietem cellulæ matricis; zoosporæ biciliatæ, encystantes extra sporangium post emissionem, e cystis emergents velut sporæ motiles. Sporæ perdurantes non observatæ.

Eurychasmidium tumefaciens (Magnus) comb. nov.

Syn. *Chytridium tumefaciens* Magnus.

Olpidium tumefaciens (Magnus) Berl. et de Toni.

Thallus filling the host cell; spherical or irregular and lobed; producing a marked enlargement of the host cell and stimulating the host to form clusters of lateral branches; becoming transformed into a single sporangium, the spherical ones 100–110 μ in diameter, the lobed examples up to 110 $\times$ 200 μ long. Zoöspores formed within the sporangium (ciliated?), upon discharge through as many as 30 discharge tubes forming a dense motionless cluster near the sporangium; encysting, the somewhat loosely disposed cysts being angular and 4 μ in greatest width; emerging from the cysts after a period of quiescence as ellipsoid, biciliated spores, 3 μ in diameter by 5 μ in length. Resting spores not observed.

This remarkable fungus which usually attacked only the nodal cells of the *Ceramium* possesses many points of interest. In its method of

[2] One curious evidence of the virulence of this fungus was noted. In a mount containing many swimming spores, a species of *Vorticella* was observed which had captured 10 or more zoöspores. The latter instead of being digested by the animal, had reversed the process and had absorbed the contents of the protozoan and were forming sporangia!

infection, developmental features, and effect on the host cell it resembles to a marked degree *Eurychasma Dicksonii* (Sparrow, 1934). However, it differs from this genus since it always remains intramatrical, since the zoöspores undergo a period of encystment outside the sporangium, not inside, as in *Eurychasma,* and since it produces adventitious lateral branches and a more marked swelling of the host cell (Plate I, Fig. 1).

The earliest stages observed in the development (Text Figs. 14, 15) showed an already well-established thallus easily distinguishable in the distended host cell by the presence of numerous irregular, refractive bodies. As growth proceeded, the disintegrated chloroplast material became more granular while the host protoplasm assumed a vacuolate character (Text Fig. 18). Coincident with the increase in size of the fungus there was a strong distention of the host cell (Text Fig. 17).

Infection is unquestionably initiated by the zoöspore, probably in the same manner as described for *Ectrogella perforans.* While actual observations on this point were lacking, there were occasionally observed instances similar to Text Fig. 16, in which the empty cyst and remains of the penetration tube were still present on the infected cell. The sequence of changes ensuing in the fungous protoplasm itself as growth proceeded were entirely similar to those described by Petersen (1905) for *Eurychasma,* including the characteristic "stade écumeux" (Text Fig. 19).

During enlargement of the host cell, a pronounced thickening of the algal wall material was noted. Also, certain adjacent nodal cells were stimulated to divide and there was produced in the vicinity of an infected cell a number of curved, stunted, lateral branches which gave a "bushy" appearance to the region (Plate I, Fig. 1). Such places could easily be detected with a hand-lens. Since the fungus was not observed to form any rhizoidal system, but was confined to a single cell, it would seem that some sort of substance was produced in infected cells which diffused to adjacent ones and stimulated them to abnormal growth.

Occasionally, infected apical cells of *Ceramium* were found with 2–6 thalli in them, probably the result of multiple infection. If these were produced by the same fungus as found in the nodal cells, it is interesting to note that here, very little hypertrophy occurred.

Unfortunately, the final stages in the formation of the zoöspores and their discharge from the sporangium through many discharge tubes were not observed, in most cases evacuation of the spores having taken place before the preparations were made. Nor could means be found to promote maturation and discharge of sporangia under observation. Hence, it is not known whether the spores emerge by ciliary action or are forced out of the sporangium. Once emerged, however, they form a dense

cloud around the infected filament, each spore being surrounded by an angular wall (Text Fig. 21). These cystospores are not connected with one another as they appear to be in the sporangium of *Eurychasma* but are loosely disposed in the medium (Text Fig. 20). After an unknown period of quiescence the protoplasm of each of these cysts emerges, probably through a pore formed in the wall, and undergoes near the empty shell a rocking and fashioning which terminates with the production of the fully formed spore. The body of the spore is somewhat elliptical, generally possessing a single, minute refractive droplet, and two cilia—the latter point being determined from a study of killed and stained material. Further observations on the orientation, length, and points of attachment of these cilia are needed.

This fungus is a true parasite, initiating and carrying to completion the destruction of the host cell unaided by other agencies. Unlike certain other marine fungi, however, it does not seem able to continue its destruction of the alga after the latter has been noticeably weakened, as, for example, by remaining under laboratory conditions for a time. The remainder of the host plant, save in the immediate vicinity of an infected cell appears unaffected by the incursion of the fungus.

6. *?Petersenia sp.* Saprophytic in *Ceramium diaphanum*, Little Harbor, Woods Hole, August 29, 1934.

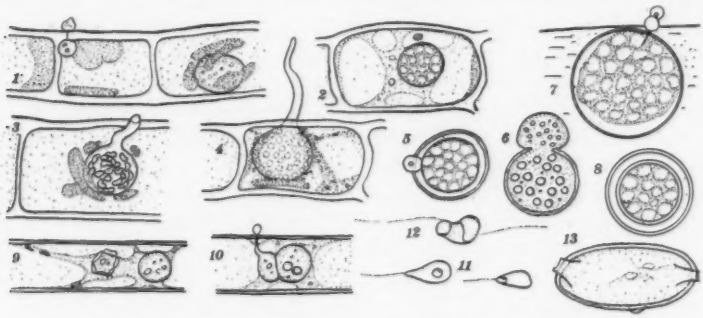
In material of *Ceramium diaphanum* which had been kept in the laboratory in an aquarium for several weeks, there was found a "Pleotrachelus"-like fungus, probably to be regarded as a saprophyte in the alga. In contrast to the previously described fungus on this alga, the present form was found only in the internodal cells and in further distinction was never observed to produce any hypertrophy of the host.

One to many thalli of varying size (20–96 μ in diameter) were formed in a single internode (Plate II, Fig. 4). While maintaining in general a spherical shape, these thalli occasionally assumed irregular forms, though no lobed examples were found.

The mature sporangium was characterized by its relatively large size, attaining frequently a diameter of 96 μ or more, although occasional specimens measured only 20–25 μ. At maturity up to ten or more narrow, distally tapering, radiating discharge tubes (about 7 μ in diameter) were formed (Plate II, Fig. 3) which bored through the cell wall of the alga. Zoöspores and resting spores were not observed, and hence the exact generic disposition of the fungus awaits the results of further investigations. If, as is probable, the swarmers are found to be biciliate, affinity with *Olpidiopsis* and more especially *Petersenia* would be indicated.

7. *?Petersenia sp.* Parasitic in eggs of a microscopic animal (rotifer?), Sheep Pen Cove, Woods Hole, August, 27, 1934.

Adherent to filaments of *Ceramium diaphanum* there was an abundance of eggs of a microscopic animal, probably belonging to the *Rotiferæ*, members of which were swarming in the mount. Some of these were parasitized by a fungus allied to *Petersenia* and *Olpidiopsis*. The thallus occupied the whole of the interior of the egg and at maturity was transformed into a single sporangium 50 μ long by 20 μ in diameter. This was provided with 1–3 broad (8 μ in diameter), short,



TEXT FIGS. 1–13. Figs. 1–8, *Petersenia Andréei;* Fig. 1, early stage in the formation of the thallus in *Ectocarpus*, the collapsed cyst of the infecting zoöspore and the penetration tube still present, a more mature thallus is seen in the right-hand cell, × 376; Fig. 2, more mature thallus containing characteristic refractive bodies, × 376; Fig. 3, mature, unopened sporangium showing some of the zoöspores which are actively moving within it, × 376; Fig. 4, mature sporangium with long extramatrical discharge tube, × 376; Fig. 5, mature resting spore with male cyst adherent to it, × 600; Fig. 6, conjugation of thalli previous to resting spore formation, × 525; Fig. 7, immature resting spore with two cystospores, which have apparently conjugated, attached by a penetration tube to it, × 760; Fig. 8, mature resting spore showing interpretation of wall structure, × 600. Figs. 9–11, (?)*Olpidium sphacellarum;* Fig. 9, young, spherical thallus in hair of *Sphacelaria*, × 525; Fig. 10, young thallus attached to nucleus of host cell, the cyst of the infecting zoöspore and penetration tube still persistent, × 525; Fig. 11, interpretation of two zoöspores, free-hand. Fig. 12, *Petersenia Andréei*, interpretation of zoöspore, free-hand. Fig. 13, *Petersenia sp.* in egg of rotifer, discharged sporangium containing a few zoöspores, × 376.

discharge tubes (Text Fig. 13). The actual formation and discharge of the zoöspores was not witnessed, although a number of these bodies which had failed to emerge were observed within the sporangia. These were reniform, of the "laterally biciliate" type, 4 μ long by 2 μ in diameter, and were very similar to those formed by *Pythium*.

No bacterial or other biological agencies appeared to assist in the destruction of the eggs. It is hoped that more material of this fungus

can be found in order that a further study of its method of infection, as well as the formation and discharge of the zoöspores can be made. From the ciliation of the spores there seems little question of its relationship to *Petersenia.*

8. *Petersenia lobata* (Petersen) Sparrow. Parasitic on *Callithamnion roseum,* growing on *Chorda filum,* dredged from between East Chop and West Chop Lighthouses, Vineyard Haven, August 22, 1934; Sheep Pen Cove, Woods Hole, August 23, 1934.

This peculiar fungus was abundant in certain tufts of the host, but it was never observed to attack all the plants on a single *Chorda.* Cells near the base of the tuft were most frequently infected.

The predominating type of thallus found in this material was cylindrical with rounded ends, although there occurred numerous lobed examples, often of very bizarre appearance (Plate II, Figs. 1, 2).

A morphological and taxonomic discussion of this fungus has recently been given by the writer (1934) and since no unusual features were displayed by the American material, it will not be repeated here.

In the early stages of invasion of the host cell, the parasite is unassisted by other organisms. However, in old infections, an abundance of bacteria and Protozoa are always found and it seems probable that the fungus initiates the destruction of the host cells, being aided in later stages by these other agencies.

9. *Petersenia* (*Olpidiopsis*) *Andréei* (Lagerheim) comb. nov.
Syn. *Pleotrachelus Andréei* Lagerheim, 1899.
Pleotrachelus ectocarpi Jokl, 1916.

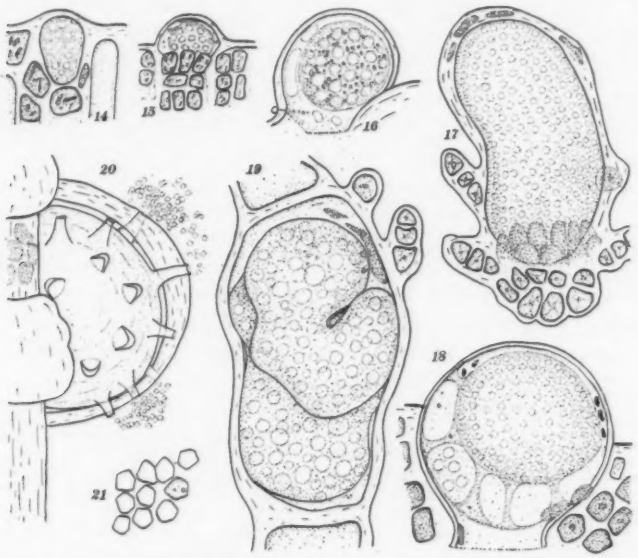
?Parasitic in *Ectocarpus siliculosus,* dredged off Gay Head, Vineyard Sound, July 16, 1934; Great Harbor, Woods Hole, August 24, 1934.

Frequent examples of extensive infection of host plants were found in the material from Gay Head, and quite commonly large fronds were observed with nearly every cell showing evidence of fungous activity. Such plants were easily recognized by their slightly greener appearance. So extensive was the infection that it could not be determined whether the fungus was the primary cause of the destruction of the alga, or whether it was only a saprophyte.

There was little doubt as to the method of entrance of the fungus. Numerous examples similar to Text Fig. 1 were observed which clearly showed that the zoöspore, after coming to rest on the surface of the host, had gained entrance by the formation of a penetration tube through which the content of the spore passed into the interior. It was the disorganized condition of the host cell contents even in early stages

of infection which seemed to indicate that the fungus was probably only weakly parasitic or even saprophytic.

Further development involved the enlargement of the thallus (Text Fig. 2), accompanied by a gradual disintegration of the host contents. One to three thalli were usually formed in a single cell. The mature sporangia lay loosely in the host cell and were generally spherical and 18–20 μ in diameter (Text Fig. 4). A single discharge tube, 4–5 μ in



TEXT FIGS. 14–21. *Eurychasmidium tumefaciens.* Figs. 14, 15, early stages in the formation of the thallus in *Ceramium diaphanum*, × 225; Fig. 16, more mature thallus showing at the bottom of the left-hand side the remains of the infecting zoöspore and its penetration tube, × 376; Figs. 17–19, strongly hypertrophied host cells with vacuolate thalli of the fungus in them, Fig. 19 shows one of the infrequently formed lobed thalli, all × 225; Fig. 20, empty sporangium showing the many discharged tubes formed and a few of the encysted zoöspores, the latter formed a dense cloud around the sporangium, × 225; Fig. 21, cysts formed by the discharged zoöspores, from all but one of which the spore has emerged, × 760.

diameter, was formed which often extended for as much as 78 μ out into the water. Occasionally, the tube did not achieve a correct orientation, in which case it passed through a number of host cells before reaching the outside.

The zoöspores were fully formed within the sporangium by a process of progressive cleavage. After maturation, and before dissolution of

the tip of the discharge tube, they underwent a prolonged period of violent individual movement and "milling around" (Text Fig. 3). Upon the opening of the discharge tube they escaped by their own efforts from the sporangium. In their morphology, these spores (Text Fig. 12) are strikingly like those of *Pontisma* (Sparrow, 1934), being irregularly piriform, arched, 5 μ long by 3 μ in diameter, and possessing at the narrow, forward end, as well as at the broader posterior one, a strongly refractive region. As has been suggested for *Pontisma*, a broad central, transverse groove and a "doubling-over" of the ends may account for this peculiar appearance. However, because of their small size, observations on moving spores are difficult. From the more anterior part of the groove there arise two cilia which appear to be of nearly equal length and are disposed in opposite directions along the long axis of the spore. These observations confirm Petersen's (1905) brief statement that the spores are laterally biciliate.

The spores moved in an erratic manner through the water. In hanging drop cells many of them germinated, each giving rise to a single, slender, very irregular tube, often of considerable length (30 μ).

Resting spores occurred in abundance (Plate I, Fig. 8). It will be recalled that in the Danish material of what is probably the same species, the writer (1934) showed that resting spore formation was apparently preceded by a conjugation between thalli of unequal size, the contents of the smaller passing into the larger which became the resting body. In the present fungus a similar phenomenon was observed in many instances and there seems little question that the resting spores are produced as a result of a sexual act almost identical with that found in the genus *Olpidiopsis*. Communication between the two cells was affected by the formation of a pore, no evidences of a definite fertilization tube ever being found. While, in general, the two conjugating thalli were more nearly equal (12 μ, 23 μ) (Text Fig. 6) than was observed in the Danish material (17 μ, 5 μ), occasionally they differed greatly in size (20 μ, 8 μ) (Text Fig. 5).

The mature resting spore was spherical, 20–23 μ in diameter, and appeared to be surrounded by a thick wall. However, under high magnification it was seen that this apparent wall could be resolved into the following structures: an outer, very definitely thickened membrane surrounding the whole, an inner membrane of the same thickness surrounding the refractive contents, and between them, a clear zone (Text Fig. 8). Whether the spore itself is to be regarded as relatively thin-walled and resting within a larger structure, or whether all of these parts are fused into a common surrounding layer, is not clear. Further observa-

tions on the development of the resting spore will be necessary to clarify this point.

Another puzzling feature was the absence in some cases of companion cells on the mature resting spores. Either the cyst was hidden from view by the body of the spore, or it soon disintegrated, or conjugation occurred in some other manner. Some evidence in favor of the latter case is given by the condition shown in Text Fig. 7. Here, attached to an immature resting spore by a tenuous penetration tube, are the cysts of two zoöspores which apparently have conjugated. It is entirely possible that after this process the merged plasma entered the host and there formed the resting spore.

In material of *Ectocarpus* in a floating *Chorda* complex collected in Great Harbor, Woods Hole, what appeared to be germinated resting spores were found. In the process of germination, the inner wall had apparently been absorbed, a long evacuation tube formed, and the contents, undoubtedly in the form of zoöspores, had been discharged through it.

EXPLANATION OF PLATE I

All photographs were made from living material with the exception of Plate I, Figs. 3, 5, and Plate II, Figs. 4, 5, which were made from material mounted in glycerine and stained with eosine. The approximate magnifications are given in each instance.

FIG. 1. *Eurychasmidium tumefaciens*, parasitic in *Ceramium diaphanum*. Two hypertrophied cells are shown; the right-hand filament has produced lateral branches in response to the incursion of the fungus. The indistinctness of the right-hand figure is due to the presence of a cloud of spores which has just been discharged from the sporangium. × 80.

FIG. 2. *Thraustochytrium proliferum*, saprophytic on *Bryopsis plumosa*. The right-hand figure is a sporangium with the spores about to be discharged; the highly refractive body at the base of the sporangium is the fundament of the new sporangium which will develop after discharge of the spores. The left-hand figure may possibly be an immature resting spore. × 380.

FIG. 3. (?)*Olpidium sphacellarum*, parasitic in *Sphacelaria cirrhosa*. An empty sporangium with two lateral, oppositely placed discharge tubes. × 330.

FIG. 4. *Sirolpidium bryopsidis*, in *Bryopsis plumosa*. Typical olpidioid thalli formed from the fragmentation of an originally filamentous thallus. × 380.

FIG. 5. *Pontisma lagenidioides*, saprophytic in *Ceramium diaphanum*. Habit of the segmented thallus in the alga. × 380.

FIG. 6. *Protomyxa*-like animal parasitic in *Bryopsis*. The delicate pseudopodium of the animal bearing a large spherical and two small, fusiform enlargements may be seen extending from the top to the bottom of the photograph. × 380.

FIG. 7. *Sirolpidium bryopsidis* in *Bryopsis*. Thalli after 24 hours exposure to dripping sea-water. Compare with Fig. 4, which is under completely submerged conditions. × 380.

FIG. 8. *Petersenia Andréei*, parasitic (?) in *Ectocarpus*. Resting spores in cells of filament. × 250.

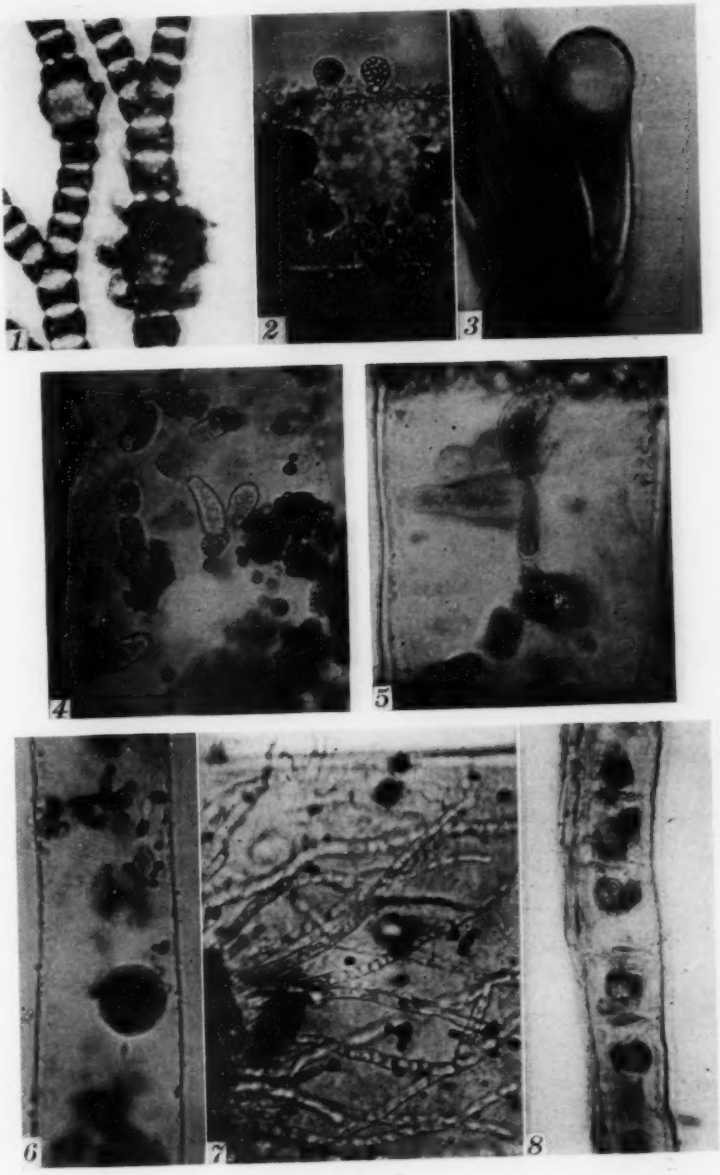


PLATE I

While a *Protomyxa*-like organism was abundant in certain cells of the *Ectocarpus*, the parts of the host occupied by the fungus were free from the animal, and, so far as could be determined, from other biological agents of disintegration as well.

10. (?)*Olpidium sphacellarum* Kny. Parasitic on *Sphacelaria cirrhosa*, Pine Island, Woods Hole, June 24, 1934.

This fungus was found in only one collection of the alga, although a search was made for it throughout the time the host was available. It attacks chiefly the apical cells of the lateral branches (sporangia?) and the colorless setæ. The rest of the plant appears immune and is not visibly affected by the presence of the fungus.

Early stages in the development were found, particularly in the colorless setæ. The infecting unit is the zoöspore, which comes to rest on the host, encysts, and produces a tenuous tube which penetrates the algal wall and through which the contents of the spore are conducted into the cell. In many cases the young thallus attaches itself to the nucleus of the host cell (Text Fig. 10). It then gradually enlarges into a spherical (Text Fig. 9), or often cylindrical structure with rounded ends. No hypertrophy is evident. In infected lateral branches (sporangia?), however, a pronounced swelling occurs (Plate I, Fig. 3, Plate II, Fig. 5). The disintegration of the cell contents is almost complete and there is generally left only a mass of irregularly-shaped, colorless granules. The mature sporangia are nearly spherical and 35–45 μ long by 30–35 μ in diameter. One, or occasionally 2–3 short, stout tubes, 8–10 μ in diameter, are formed through which the mature zoöspores are discharged. Before these are ejected they undergo a period of active swarming, eventually being liberated very rapidly at the dissolution of the tip of the discharge tube. They are minute, being 4 μ long by about 2 μ in diameter, and have a somewhat piriform body (Text Fig. 11), near the broader end of which there is a prominent refractive globule. The position of this globule may change while the spore is motile. Com-

---

EXPLANATION OF PLATE II

FIGS. 1, 2. *Petersenia lobata*, in *Callithamnion roseum*. Two thalli, showing the peculiar lobing sometimes encountered. × 260.

FIG. 3. *Petersenia sp.*, saprophytic in *Ceramium diaphanum*. An empty sporangium with three discharge tubes visible. × 260.

FIG. 4. Same fungus, showing the spherical, immature thalli and several discharged sporangia. × 260.

FIG. 5. (?)*Olpidium sphacellarum*, parasitic in *Sphacelaria*. Two thalli, one in the colorless hair, the other in the hypertrophied tip of the lateral branch. × 330.

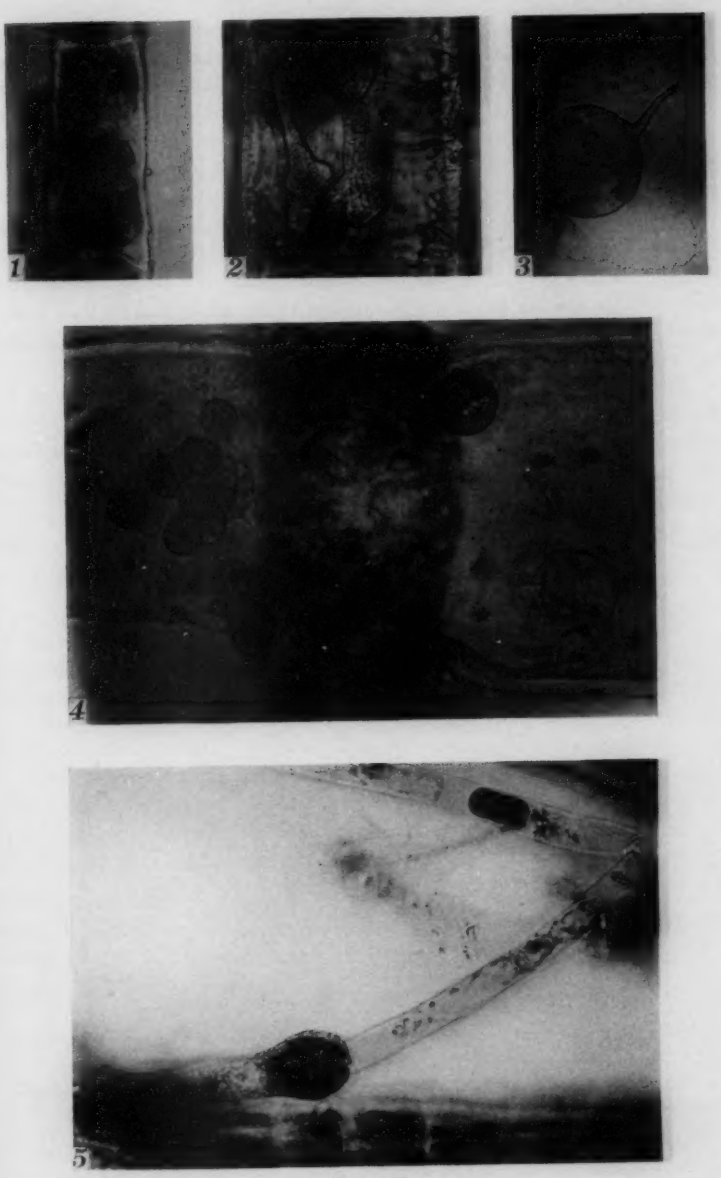


PLATE II

plete data on the ciliation of these spores were not obtained. It is certain that they possessed a very prominent, forwardly directed cilium which appeared to remain nearly stationary during the period of motility. It is probable that another, rapidly vibrating, possibly shorter, posteriorly directed cilium was also present but escaped detection. The zoöspore exhibited a very peculiar type of movement which was characterized by periods of even, forward progression, alternating with sudden stops and change of direction. There appeared to be practically no rotation of the body during movement.

The fungus was a true parasite, invading only the healthy cells of the host and accomplishing alone the disintegration of the contents. With the decline of the alga under laboratory conditions no further fungous activity was noted.

While considered a species of *Olpidium,* it is evident from what is known of the ciliation of the spores that this fungus bears only a superficial resemblance to this genus. If, as is highly probable, the zoöspores are found to be biciliate, it should be placed near *Olpidiopsis* and *Petersenia.*

11. *Pontisma lagenidioides* Petersen. (?)Saprophytic in the internodal cells of *Ceramium diaphanum,* Little Harbor, Woods Hole, July 8, 1934.

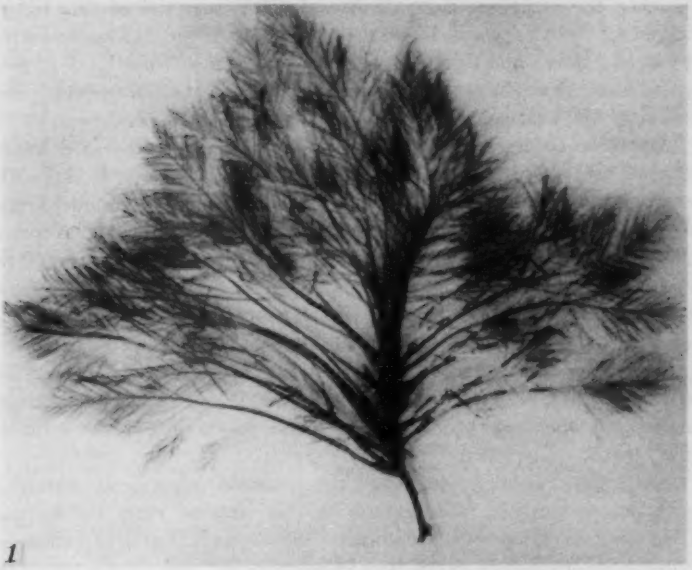
Since details of the morphology of this species have been given in a recent paper (Sparrow, 1934), they will not be repeated here. No significant differences from the Danish material were apparent, except for the fact that the Woods Hole fungus (Plate I, Fig. 5) could at the most be called only weakly parasitic on the *Ceramium.* Indeed, only after the alga had been maintained in the laboratory for several weeks did the fungus appear in appreciable quantity. While occasional thalli were formed in apparently living cells free from other organisms, they occurred more commonly in dead and somewhat desiccated algæ along with bacteria and Protozoa.

12. *Sirolpidium bryopsidis* (de Bruyne) Petersen. Parasitic in *Bryopsis plumosa,* Woods Hole, throughout the summer, 1934; June–July, 1935; *Cladophora sp.* (saprophytic?), Woods Hole, July 1, 1935.

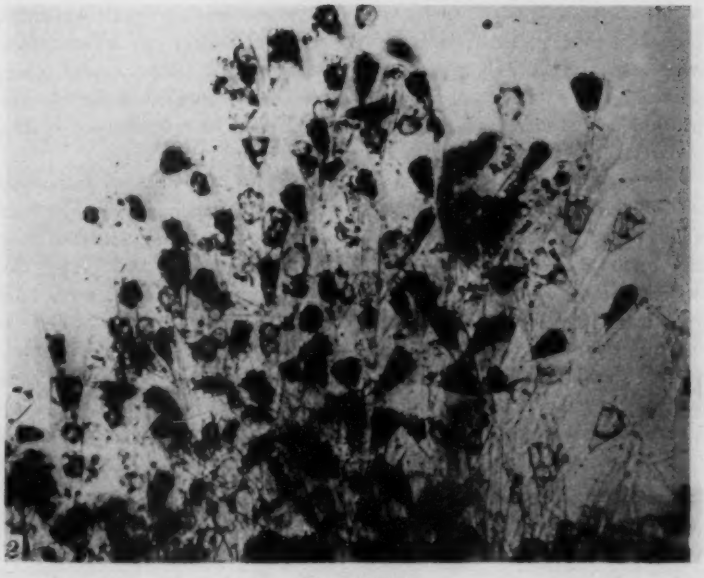
EXPLANATION OF PLATE III

FIG. 1. Plant of *Bryopsis plumosa* infected with *Sirolpidium bryopsidis.* The blackened areas caused by the fungus may be seen particularly well along the axes of the lateral branches. About natural size.

FIG. 2. *Ectrogella perforans,* parasitic in *Licmophora abbreviata.* A stand of the diatoms heavily infected with the fungus. Infected cells may be distinguished by the light, spherical sporangia of the *Ectrogella* within them. × 260.



1



2

PLATE III

As in the preceding case, the Woods Hole material of this fungus showed no morphological differences from that found in Danish waters (Sparrow, 1934) and this phase of the organism will not be dealt with again here. It might be noted, however, that the zoöspore effects penetration of the host cell exactly as in (?)*Olpidium sphacellarum*.

Macroscopically, heavily infected plants may be recognized by the presence of blackened areas along the fronds (Plate III, Fig. 1). The general aspect of the fungus in the alga is shown in the photomicrograph (Plate I, Fig. 4). While early in the course of the infection the fungus undoubtedly acted alone in the entering and the breaking down of the algal contents, in later stages it was often accompanied by swarms of bacteria and Protozoa. Mention has been made already (p. 238) of the peculiar Protomyxa-like animal which often competed with the fungus. Indeed, in some cases this association was so constant that had this animal not been entirely absent in the Danish material, it might easily have been supposed to be a phase in the life history of the *Sirolpidium*.

Since this fungus, by reason of the somewhat filamentous nature of its thallus, seemed the most suitable of these marine fungi for the purpose, attempts of a very preliminary nature were made to culture it. It had been previously noted that if infected *Bryopsis* plants were put in close contact with air, such as allowing water to drip over them rather than to submerge them entirely, the fungus underwent a remarkable transformation in body structure. Instead of the formation of typical olpidioid thalli (Plate I, Fig. 4), there were formed definitely filamentous, ramifying hyphæ (Plate I, Fig. 7). The resemblance of these to the hyphæ of the higher Phycomycetes was very striking.

Pieces of *Bryopsis* containing such hyphæ were placed in various nutrient solutions to determine whether or not further vegetative growth could be induced. Dextrose, mannose, galactose, and fructose were used in Seitz-filtered sea-water solutions of 0.5 per cent and 1.0 per cent and maintained at 19–20° C. Certain protein solutions were tried but fouled too rapidly from bacterial action to be of use. The amount of extramatrical growth was used as a criterion for the efficacy of the particular solution. Suitable controls in Seitz-filtered sea water alone were also set up. Since it was soon found that only the dextrose had a pronounced effect on growth, further solutions of this sugar were made up to determine its most favorable concentration. The results of these preliminary trials are given in Table I.

From these data it can be seen that the lower concentrations of sugar were most effective in stimulating extramatrical growth, whereas the higher concentrations, while they were not lethal to the fungus, seemed

to have been definitely inhibitory. Attempts were then made to culture *Sirolpidium* on a solid medium by placing bits of the alga containing extramatrical growth on 0.5 per cent and 1.0 per cent dextrose solutions to which had been added either 0.5 per cent or 1.0 per cent agar. Further growth could not be induced. Studies on the cultivation of *Sirolpidium* are being continued with the hope that from the data obtained from this particularly favorable fungus a method can be devised for getting a number of these organisms into culture. If this can be accomplished, then specific information can be obtained regarding the nature of the activities carried on by these marine fungi.

TABLE I

Extramatrical growth of Sirolpidium in dextrose solutions at 19–20° C.

Solution per cent	Effect on growth			
	24 hours	48 hours	56 hours	80 hours
0.0 (control)	Practically none.	No change.	No change.	No change.
0.5	Excellent growth, up to 100 μ.	Up to 197.5 μ.	No change.	No change; mycelium fragmenting.
1.0	Good growth; up to 62–87 μ.	Up to 112 μ.	No change.	No change; mycelium fragmenting.
1.5	Very little; up to 20–30 μ.	Up to 100–137 μ.	Up to 140 μ.	Up to 175 μ.
2.0	None.	None.	Up to 20 μ.	No change.

13. *Chytridium polysiphoniæ* Cohn. Parasitic on *Polysiphonia fibrillosa,* Woods Hole, August 27, 1934; saprophytic on *Ceramium rubrum,* Woods Hole, August 24, 1934.

Details of the morphology and reproduction of this species are given in a previous paper (1934) by the writer. On *Polysiphonia* (Text Fig. 31), the Woods Hole fungus was an undoubted parasite, whereas it was only saprophytic on *Ceramium,* as was indicated by the whitened and disorganized contents of the alga. Bacteria and Protozoa were abundant in cells of the latter alga, but were absent in infected cells of the *Polysiphonia.*

14. *Chytridium megastomum* Sparrow. Parasitic on *Ceramium diaphanum,* Woods Hole, August 24, 1934.

No morphological differences from the Danish fungus (Sparrow, 1934) could be observed in this material. The sporangia occurred in great numbers on all parts of the alga; bacteria and Protozoa were, how-

ever, absent. An interesting feature of the parasitism of this fungus was the fact that the *Ceramium* appeared at the time of observation unaffected by the presence of the *Chytridium*. It seems probable, however, that even though the fungus itself did not exert a marked effect on its host, the innumerable punctures in the cell wall of the alga laid it open to entrance and attack by bacteria, and later, Protozoa. Such a sequence did occur when infected plants were maintained in a laboratory aquarium, but this does not necessarily indicate that such also takes place under natural conditions.

15. *Pleolpidium* (*Rozella*) *marinum* n. sp. Parasitic on *Chytridium polysiphoniæ*, Woods Hole, August 27, 1934.

In material of *Chytridium polysiphoniæ* parasitic on *Polysiphonia fibrillosa*, occasional sporangia were observed which were of larger diameter (30–45 μ) and possessed a thicker wall and more oleaginous contents than others. In several instances these were seen to discharge motile bodies through 1–3 pores (Text Fig. 33). The spores were minute, 3 μ long by 2 μ in diameter, ellipsoid, posteriorly uniciliate, and in contrast to those of *C. polysiphoniæ*, lacked a refractive oil droplet in their content. After a period of swarming, certain ones settled down on the surface of the sporangia of *C. polysiphoniæ* and were observed to produce a fine penetration tube (Text Fig. 32). Further development was not witnessed.

While this fungus might at first sight be considered a multiporous species of *Rhizophidium*, it is in reality a parasite of the *Chytridium*, closely allied to certain fresh-water species usually placed in the genus *Pleolpidium*. Reasons for referring all these species to Cornu's genus *Rozella* have been stated in a previous paper (Sparrow, 1936). Since no marine representatives of this genus have heretofore been described, the fungus is considered a new species, and is termed *Pleolpidium* (*Rozella*) *marinum* n. sp.

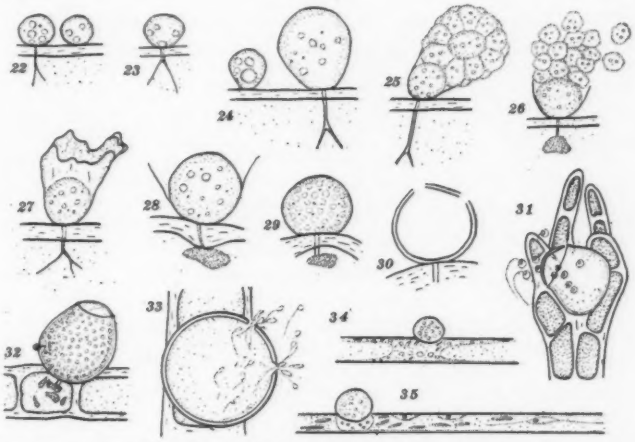
Pleolpidium marinum n. sp. Sporangium spherical, completely filling that of the enlarged host plant; 30–45 μ in diameter, at maturity forming 1–3 pores through which the zoöspores are discharged; zoöspores ellipsoidal, 3 μ long by 2 μ in diameter, posteriorly uniciliate, aguttulate. Resting spores not observed.

Parasitic on *Chytridium polysiphoniæ*.

P. marinum sp. nov. Zoösporangium globosum, cellulam matricis distentam omnino implens, 30–45 μ dia., maturitate 1–3 ostiolatis præditum per quæ zoösporæ emittuntur; zoösporæ ellipsoideæ, 3 μ long. v. 2 μ dia., posteriore uniciliatæ, aguttulatæ. Sporæ perdurante non observatæ.

16. *Rhizophidium discinctum* Petersen. Parasitic(?) on *Ceramium diaphanum*, Woods Hole, August 17, 1934.

A fungus probably identical with that found by Petersen (1905) on *Spongomorpha* and *Acrosiphonia* in Scandinavia was found attacking the nodal cells of the *Ceramium* which had been maintained in a laboratory aquarium for a week. It is probable that the fungus was only weakly, if at all, parasitic on the alga.



TEXT FIGS. 22–35. Figs. 22–28, *Thraustochytrium proliferum*, on *Bryopsis plumosa*, all × 760. Figs. 22–24, various stages in the development of the sporangium; Fig. 25, mature sporangium showing basal body which will become the fundament of the secondary sporangium; Fig. 26, discharge of the zoöspores after the bursting and deliquescence of the upper part of the sporangial membrane; Fig. 27, discharged primary sporangium with secondary sporangium beginning to enlarge; Fig. 28, more mature secondary sporangium. Figs. 29–30, *Rhizophidium discinctum* on *Ceramium diaphanum;* Fig. 29, sub-spherical immature sporangium, × 376; Fig. 30, empty sporangium showing angular aspect and thick wall, × 376. Figs. 31–32, *Chytridium polysiphoniæ;* Fig. 31, discharging sporangium on *Polysiphonia*, × 376; Fig. 32, mature sporangium attacked by a zoöspore of *Pleolpidium* (Rozella) *marinum* × 376. Fig. 33, *Pleolpidium marinum*, Fig. 33, discharging sporangium of the fungus in *Chytridium polysiphoniæ*, × 376. Figs. 34–35, *Rhizophidium globosum*(?) on *Rhizosolenia sp.;* Fig. 34, sporangium with peglike intramatrical tube, × 376; Fig. 35, sporangium apparently attached to nucleus of host, swarm spores of *Labyrinthula sp.* also attacking diatom, × 376.

The sporangia are at first spherical or hemispherical (Text Fig. 29), but at maturity, because of the formation of one or two laterally-placed discharge papillæ, become more angular. In some cases the upper part of the sporangium may be very definitely flattened. A further characteristic is the thickness of the sporangium wall, a feature very evident

in discharged sporangia (Text Fig. 30). Because of the density of the host contents and especially because of the thickness of the algal wall, observations on the nature of the intramatrical system were difficult. So far as could be determined, this structure appeared to consist of a rather stout, unbranched tube. However, it is possible that it branched deeper in the host. Because of this uncertainty it is probably better to keep the fungus in the genus *Rhizophidium* than to transfer it to *Phlyctidium*, where, because of the unbranched rhizoid, it might be placed.

Martin's fungus (Martin, 1922), termed by him *Rhizophidium polysiphoniæ* (Cohn) Petersen, is probably referable to this species.

17. *Rhizophidium globosum* (Br.) Schröt.(?). Parasitic on *Bryopsis plumosa*, Woods Hole, August 8, 1934; saprophytic on *Rhizosolenia spp.*, Woods Hole, August 16, 1934.

The sporangia of the form on *Bryopsis* were spherical, 13–18 μ in diameter, and were attached to the host cell by a delicate, scarcely perceptible, branched, intramatrical rhizoidal system. In contrast to *Rhizophidium discinctum*, the even contour of the sporangium was maintained at maturity.

The fungus attacked only healthy cells of the *Bryopsis*. While the parasite at the time of observation did not appear to have any noticeable effect on the host, it undoubtedly paved the way for the more destructive activities of later invaders.

What may possibly be a closely related form was found in some abundance on cells of *Rhizosolenia spp.* dredged from the bottom of Great Harbor during a heavy "flowering period" of diatoms. The sporangia (Text Figs. 34, 35) were globose to sub-globose, 9–12 μ in diameter, and were generally found singly, attached to the mid-region of the diatom. A single, delicate rhizoid penetrated the alga and possibly attacked the nucleus. Discharge of the zoöspores was not observed and hence, the possibility that we are dealing with a species of *Chytridium* cannot be overlooked.

The fungus on *Rhizosolenium* was found only on obviously disintegrating diatoms, generally in company with a species of *Labyrinthula* (Text Fig. 35) and a *Protomyxa*-like animal. Flagellates were also frequently observed in such cells. No bacteria could be demonstrated in diatoms having both fungus and *Labyrinthula*, or fungus alone, in them.

Both of these fungi are only tentatively referred to the morphologically similar species inhabiting fresh-water algæ.

18. *Thraustochytrium proliferum* n. gen. n. sp. Saprophytic on *Bryopsis plumosa,* July 30, 1934; *Ceramium diaphanum,* August 17, 1934; Woods Hole, Mass.

Thraustochytrium n. gen. Spore upon coming to rest on the host cell encysting and producing an intramatrical rhizoidal system; body of the spore enlarging and becoming the sporangium; spores formed within the sporangium, liberated by the bursting and dissolution of the whole distal portion of the sporangial wall; spores non-ciliate when liberated; new sporangia formed by a process of internal proliferation. Resting spores not observed.

Thraustochytrium gen. nov. Spora cellulæ matricis insidens, encystans ac systema intramatricale rhizoideum proferens; corpus sporæ se maius faciens atque sporangium exsistens; sporæ intra sporangium factæ et eruptione dissolutioneque totius partis distalis parietis sporangialis liberatæ; sporæ non ciliatæ quo tempore liberatæ; nova sporangia ratione proliferationis internæ formatæ. Sporæ perdurantes non observatæ.

T. proliferum n. sp. Sporangia smooth, piriform, very thin-walled, resting on the narrow base; 15.6–18 μ high by 10–13 μ in diameter; rhizoid branched or unbranched, of limited extent; zoöspores somewhat angular at discharge, later becoming spherical, 4 μ in diameter.

T. proliferum[3] sp. nov. Sporangia piriformia, angustæ basi insidentia, levia, pariete tenuissimo, 15.6–18 μ longa, 10–13 μ dia., rhizoidea ramosa aut non ramosa extensionis parvæ; zoösporæ angulosiores quo tempore emissæ, serius sphæroideiores exsistentes, 4 μ dia.

The developmental features of this interesting fungus (Text Figs. 22–24) (Plate I, Fig. 2) are in the main similar to those of *Chytridium* and *Rhizophidium.* However, in the *Thraustotheca*-like discharge of the zoöspores and in the proliferation of the sporangium it possesses two very distinctive characteristics. A further peculiarity is its method of internal proliferation. After the cleavage of the zoöspores there can generally be seen a larger protoplasmic unit occupying the narrow, basal portion of the sporangium (Text Fig. 25). Upon the bursting and deliquescence of the upper part of the sporangial wall by the enlarging zoöspores (Text Fig. 26), these bodies float away leaving only the basal protoplasm (Text Fig. 27). This enlarges (Text Fig. 28) and in time becomes a sporangium. Thus, in contrast to the proliferating sporangia of *Saprolegnia, Pythiomorpha,* etc., the fundament of the secondary sporangium of *Thraustochytrium* is delimited at the time of zoöspore cleavage in the primary body.

There can be no question of the non-ciliate character of the zoöspores at the time of discharge. However, in mounts containing the fungus occasional posteriorly uniciliate zoöspores have been observed which are

[3] I am indebted to Prof. R. C. Nemiah for the Latin diagnoses of new genera and species given in this paper.

of the same size as those discharged from the sporangia. Since no other chytridiaceous fungi were found on that particular material of *Bryopsis*, it is entirely possible that after a period of relative quiescence, the spores became ciliate.

Thraustochytrium differs from its closest relative *Rhizophidium*, in the internal proliferation of its sporangia, in its method of zoöspore discharge, and possibly in the possession of non-ciliate spores.

The fungus was found only on very disintegrated plants of *Bryopsis*, where it sometimes occurred with *Sirolpidium*, swarms of bacteria, and Protozoa.

Discussion

If we turn to a broader consideration of the organisms discussed in this paper, certain points of more general interest are apparent.

Perhaps the foremost of these is the fact that fungi are unquestionably demonstrated to be present in our marine waters. Although their occurrence has been recorded almost wholly from the vicinity of Woods Hole, it is highly probable that they will be found generally distributed in the littoral regions of the United States. While the number of species is as yet small, this does not necessarily imply that they are actually rare, either in kind or number. One need only to compare the bibliographies of fresh-water and marine fungi to appreciate the small amount of investigation that has thus far been accorded the latter group. Undoubtedly, more intensive work will reveal many hitherto unrecorded species.

These preliminary investigations would seem to indicate that the fungous flora in the vicinity of Woods Hole does not differ materially from that found on the other side of the Atlantic. Seven of the 13 fungi collected by the writer in the littoral of the Kattegat have been found at Woods Hole. Further indications of a probably widespread distribution of marine fungi are to be found in *Sirolpidium*, now known from the Gulf of Naples, the Kattegat (Denmark), and Woods Hole, in *Petersenia Andréei*, known from King Charles Land (Arctic), Greenland, Denmark, the Adriatic, and Woods Hole, and in *Eurychasmidium*, known from the North Sea near Edinburgh, the Baltic, and Woods Hole. It is also probable that a fungus from the Adriatic termed *Ectrogella Licmophoræ* by Scherffel (1925) is referable to *E. perforans* Pet., in which case that parasite would now be known from the Adriatic, the Baltic, and the vicinity of Woods Hole. The species of *Labyrinthula* reported by Renn (1934) as being associated with the wasting disease of *Zostera* has been observed by the present writer in diseased material of this plant from the Kattegat (1933), from Woods Hole (1934), and

from Plymouth Harbor, England (1935). The organisms from these localities were morphologically indistinguishable. Future work will undoubtedly further corroborate this marked similarity of the floras which is even now so apparent.

Another point of interest is the abundance in the sea of so-called chytridiaceous fungi which possess biciliate zoöspores. While those inhabiting fresh-water algæ are, in the main, uniciliate species (true chytrids), in marine algæ, biciliate forms are more frequently found. From experience in investigating both types of habitat, the writer is inclined to believe that this is a real difference and not an apparent one resulting from the disproportionate amount of work thus far done in the two groups.

As to the question of the abundance of any one species and of the extent to which by its parasitic activities it actually limits the occurrence of a "producer," very little is now known. Of the marine fungi observed by the writer, *Ectrogella perforans* produces the most extensive infection. Indeed, a closer examination of this disease might reveal that, in some instances, it is responsible for the partial or complete disappearance of the diatom from a locality. While it is true that the two diatoms most susceptible to this fungus are littoral types (*Licmophora abbreviata, Striatella unipunctata*), it is not known whether similar epidemics may be produced by *Ectrogella* among pelagic species. In this connection, certain of the peculiar rhythms of "flowering periods" of pelagic diatoms which have been noted in the past and which cannot be attributed to changing physical factors might well be considered from the standpoint of the presence of parasites. Due to the importance of diatoms in their rôle of "producers" in the sea, such a disease as that caused by the *Ectrogella* should be examined in all its aspects, even though at the moment it appears to be confined to littoral algæ. Further, it would be well in the future to give closer attention to the examination of plankton samples for evidences of parasitic organisms, especially during the decline of a "flowering period."

In the formation of "marine humus," as Waksman (1933) has so aptly termed certain types of bottom deposits, future work may show that fungi as well as bacteria play a significant part. It will be recalled that in bottom samples taken in Great Harbor, Woods Hole, during a heavy flowering period of diatoms, two types of fungi were found in some abundance on *Rhizosolenia spp.* (*Labyrinthula* and *Rhizophidium*). Both of these "reducers" were attacking diatoms in all stages of decomposition and many of the algæ must, from their well-organized contents, have either recently fallen to the bottom or had been infected while still alive. Efforts were made to determine whether or not infected

diatoms could be observed in samples from surface tows. While such indeed were found, from the nature of the waters in the vicinity of Woods Hole, subject as they are to strong tidal forces, these cannot be taken as proof of infection under pelagic conditions. It is just as possible that they were swept from the bottom as a result of tidal action. Work on the frequency of occurrence of these bottom fungi will be necessary before any estimate can be made of the extent to which they contribute to the general cycle of decomposition in the "humus."

In considering these fungi from the standpoint of their parasitism, we find a wide variation among them. Certain ones (*Ectrogella perforans, Eurychasmidium tumefaciens, Petersenia sp.* in rotifer eggs, (?)*Olpidium sphacellarum, Pleolpidium marinum, Chytridium megastomum* and *Rhizophidium* (?)*globosum* on *Bryopsis*) were true parasites and attacked, unassisted by other organisms, healthy host plants. None of these was ever observed living saprophytically. Other species (*Rhizophidium discinctum* and *Petersenia Andréei*) were only doubtfully parasitic, whereas, *Labyrinthula Chattoni*(?), *Thraustochytrium proliferum, Rhizophidium globosum*(?) on *Rhizosolenia*, and *Petersenia sp.* on *Ceramium* were saprophytes and were found only on dead algal cells. Until it can be shown experimentally that the *Labyrinthula* on decaying diatoms is the same as that parasitic on *Zostera*, it seems better to regard the two as distinct species. *Sirolpidium bryopsidis* and *Chytridium polysiphoniæ* were facultative saprophytes. It might be interpolated here that, while *Pontisma lagenidioides* was observed to be only saprophytic in the vicinity of Woods Hole, in Denmark the fungus was found to be parasitic as well, and hence it should be classed with *Sirolpidium* and *Chytridium polysiphoniæ* as a facultative saprophyte.

It is a pleasure to acknowledge at this time the continued interest of Dr. S. A. Waksman in the progress of these investigations. The writer is also indebted to members of the Botanical Staff of the Marine Biological Laboratory, particularly to Dr. H. Croasdale, for bringing in material of certain algæ.

Summary

In the foregoing paper are described fifteen species of phycomycetous marine fungi, two myxomycetes, and a *Protomyxa*-like protozoan, all collected in the vicinity of Woods Hole, Mass. Included in these are two new genera and two new species. With one exception, the fungi were either parasites, facultative saprophytes, or saprophytes on various species of marine algæ. One was found parasitizing the eggs of a microscopic animal, probably a rotifer. The results of preliminary at-

tempts to culture *Sirolpidium bryopsidis*, a facultative saprophyte of the green alga, *Bryopsis plumosa*, are reported. None of these fungi, with perhaps the exception of *Rhizophidium discinctum*, has heretofore been known from the United States. The existence of true marine fungi, their work as one of the several types of "reducers" in the sea, and their possible importance in the formation of marine humus are discussed in particular.

BIBLIOGRAPHY

CIENKOWSKI, L., 1867. Ueber den Bau und der Entwickelung der Labyrinthuleen. *Arch. f. mikr. Anat.*, **3**: 274.

DANGEARD, P. A., 1932. Observations sur la famille des Labyrinthulées et sur quelques autres parasites des Cladophora. *Le Botaniste*, **24**: 217.

JEPPS, M. W., 1931. Note on a marine Labyrinthula. *Jour. Mar. Biol. Assn.*, **17**: 833.

MARTIN, G. W., 1922. Rhizophidium polysiphoniæ in the United States. *Bot. Gaz.*, **73**: 236.

PAPENFUSS, G. F., 1935. Alternation of generations in Ectocarpus siliculosus. *Bot. Gaz.*, **96**: 421.

PETERSEN, H. E., 1905. Contributions à la connaissance des Phycomycètes marins (Chytridineæ Fischer). *Oversigt K. danske vidensk. Selsk. Forhandl.*, **1905**: (5) : 439.

RENN, C. E., 1934. Wasting disease of Zostera in American waters. *Nature*, **134**: 416.

SCHERFFEL, A., 1925. Endophytische Phycomyceten-Parasiten der Bacillariaceen und einige neue Monadinen. *Arch. f. Protist.*, **52**: 1.

SPARROW, F. K., JR., 1934. Observations on marine Phycomycetes collected in Denmark. *Dansk. bot. Ark.*, **8**: (6) : 1.

SPARROW, F. K., JR., 1936. A contribution to our knowledge of the aquatic Phycomycetes of Great Britain. *Trans. Linn. Soc. London* (in press).

WAKSMAN, S. A., 1933. On the distribution of organic matter in the sea bottom and the chemical nature and origin of marine humus. *Soil Sci.*, **36**: 125.

ZELLER, S. M., 1918. Fungi found on Codium mucronatum. *Publ. Puget Sound Biol. Sta.*, **2**: 121.

OCCURRENCE AND DISTRIBUTION OF THE MARINE PROTOZOAN SPECIES IN THE WOODS HOLE AREA[1]

JAMES B. LACKEY

(*From the Woods Hole Oceanographic Institution*[2])

Investigations of the marine species of Protozoa and of their regional, seasonal, and vertical distribution and numbers are few although much descriptive attention has been given to certain groups. Such are the studies of the Radiolaria collected by the Challenger and other expeditions; the work of Brady (1884) and Rhumbler (1900) on the Foraminifera; or the papers of Schaefer (1923, 1924, 1926) on the marine amœbas. The marine Mastigophora have received scant attention as a whole although Lohmann (1902) and Schiller (1926) have published important papers. The Dinoflagellata, however, have been extensively investigated, notably by such workers as Kofoid and Swezy (1921), and Lebour (1925). There is even less general work on the marine Ciliata, although the Tintinnoidea have been extensively studied by some workers, notably Jorgensen (1927), and there are descriptive papers by Fauré-Frémiet (1924) and Kahl (1928). The "Nordische Plankton" presents a survey of the marine Protozoa, and the chapter on pelagic plant life by Gran (1912) in "The Depths of the Ocean" gives a generalization of what is known with regard to the pigment-bearing forms. How little we actually know of the occurrence and functions of the Protozoa in the seas is indicated by the fact that in the book "The Marine Plankton," (Johnstone, Scott and Chadwick, 1924) the Protozoa are not considered except for the "vegetable plankton," i.e., peridinians, whereas it has been definitely shown that a considerable part of the plankton both by numbers and volume may consist of Protozoa.

They are probably more numerous and more important than is generally realized. Gran (1912) recorded more than "200,000 calcareous flagellates per liter . . . just outside the European coast bank" and Allen (1919) reported 14,450 per liter of sea water. He used the hand centrifuge to concentrate the organisms but was dissatisfied with the method, so by diluting his sea water into culture media and counting his

[1] Journal Series Paper, New Jersey Agricultural Experiment Station, Department of Water and Sewage Research.

[2] Contribution No. 65.

cultured organisms, he estimated that the population of the sample was at least 464,000 per liter, and says " The real number must be greater than this." Only about two-fifths (178,000) of this population were Protozoa, but any such number must exert a considerable influence on the other organisms in the same water. On the other hand, as Allen pointed out, his estimated number of Protozoa may have been too small since his media were not particularly favorable for the growth of these animals.

If the marine Protozoa are as abundant as these two instances indicate, they certainly merit more careful investigation as plankton components. Clemens' (1933) North American Pacific Coast check list includes 300 species; Smith (1904) noted 54 species from the Gulf of Mexico; Calkins (1902) studied 74 species from the Woods Hole area. Gran (1933) gave the numbers present and distribution of 4 species of flagellates, 22 of dinoflagellates, and 9 of ciliates from the Gulf of Maine; Cushman (1908) found 28 species of Foraminifera in the Woods Hole region; Martin (1929) listed 35 species of dinoflagellates from the New Jersey coast. Very little seasonal distribution has been studied in North American waters, but this phase has been investigated in the North Sea and the English Channel by Cleve (1899, 1900), Gran (1912), Herdman and Scott (1908–15), Lebour (1917) and others. From June first through early September, 1934, I studied the occurrence and distribution of the Protozoa of the Woods Hole Area.

Procedure

Studies were principally confined to those organisms occurring in water taken at the wharf of the Oceanographic Institution; the water is so turbulent there because of the strong tidal current sweeping through the Hole between Buzzards Bay and Vineyard Sound that this point samples both and there is hardly a difference in the vertical distribution of the plankton. Samples were also taken from the Bay and the Sound at various times with occasional samples from more distant points. Swimming and floating forms were secured by filtering or centrifuging samples of water varying from 120 cc. to 5 liters. A plankton net was often used to determine what larger species were present but was useless for counting purposes although relative numbers could be determined by examining the catch. Bottom-dwelling forms were secured by suspending traps from the float and by core sampling devices; later in the summer a small core bottom sampler was constructed.

TABLE I

The genera and species of marine Protozoa identified from the Woods Hole area

	Floating	Attached or in Debris	From Bottom Samples
CLASS			
Sarcodina			
SUBCLASS			
Rhizopoda			
ORDER			
Proteomyxa			
Labyrinthula sp.		X	
Vampyrella sp.	X	X	
Nuclearia simplex		X	
Arachnula impatiens	X	X	
Proteomyxa sp.		X	X
Biomyxa vagans		X	X
Foraminifera			
Pulvinulina menardii		X	X
Pulvinulina lateralis		X	X
Rotalia sp.		X	X
Spiroloculina sp.		X	X
Biloculina elongata		X	X
Polystomella striatopunctata		X	X
Miliolina seminulum		X	X
Miliolina circularis		X	X
Amoebaea			
Pelomyxa sp.			X
Vahlkampfia limax	X	X	X
Vahlkampfia albida	X	X	X
Amœba spp.	X	X	X
Hartmanella hyalina	X	X	X
Testacea			
Allogromia lagenoides		X	
Allogromia ovalis		X	X
Shepheardiella sp.		X	
Amphitrema sp.		X	
Pseudodifflugia gracilis		X	
Actinopoda			
Heliozoa			
Oxnerella maritima		X	
Eleorhanis sp.	X	X	
Raphidiophrys sp.		X	
Wagnerella borealis		X	
Pinnacocystis sp.	X	X	
Actinophrys sol	X	X	
Actinophrys sp.	X	X	
Clathrulina elegans	X		

	Floating	Attached or in Debris	From Bottom Samples
CLASS			
Mastigophora			
SUBCLASS			
Phytomastigoda			
ORDER			
Chrysomonadida			
Chrysapsis agilis	X	X	
Chromulina minor	X		
Chromulina mikroplankton	X		
Chromulina sp.	X		
Hymenomonas roseola	X		
Erythrymonas haltericola	X		
Kephyrion ovum	X	X	X
Ochromonas variabilis	X		
Ochromonas ludibunda	X		
Pontosphaera huxleyi	X		
Syracosphæra sp.	X		
Calyptrosphæra oblonga	X		
Mesocena sp.	X		
Dictyocha fibula	X		
Dictyocha staurodon	X		
Distephanus speculum	X		
Distephanus crux	X		
Distephanus crucis	X		
Cryptomonadida			
Cryptomonas erosa	X		
Chilomonas paramecium	X	X	
Chlorachne desmorpha	X	X	
Rhodomonas baltica	X		
Rhodomonas pelagica	X		
Rhodomonas sp.	X		
Nephroselmis marina		X	
Dinoflagellida			
Exuviælla lima	X		
Exuviælla compressa	X		
Prorocentrum dentatum	X		

TABLE I (*Continued*)

	Floating	Attached or in Debris	From Bottom Samples
CLASS			
Mastigophora (cont.)			
SUBCLASS			
Phytomastigoda (cont.)			
ORDER			
Dinoflagellida (cont.)			
Prorocentrum scutellum	X		
Prorocentrum triangulatum	X		
Prorocentrum micans	X		
Peridinium depressum	X		
Peridinium roseum	X		
Peridinium achromaticum	X		
Peridinium trochoideum	X		
Peridinium mite	X		
Peridinium pallidum	X		
Peridinium inermes	X		
Peridiniopsis asymmetricum	X		
Ceratium tripos	X		
Ceratium tripos var. *atlantica*	X		
Ceratium tripos var. *atlantica* forma *baltica*	X		
Ceratium tripos var. *atlantica* forma *neglecta*	X		
Ceratium tripos var. *subsalsa*	X		
Gonyaulax triacantha	X		
Gonyaulax scrippsiæ	X		
Gonyaulax spinifera	X		
Gonyaulax digitale	X		
Dinophysis norvegica	X		
Dinophysis rotundata	X		
Dinophysis acuminata	X		
Dinophysis homunculus	X		
Dinophysis caudata	X		
Dinophysis ovata	X		
Phalacroma roseum	X		
Phalacroma rotundatum	X		
Diplopsalis lenticula	X		
Glenodinium troichoideum	X		
Oxyrrhis marina	X		
Amphidinium longum	X		
Amphidinium operculatum	X		
Amphidinium punctifera	X		
Amphidinium crassum	X		
Amphidinium fusiforme	X		
Gymnodinium punctatum	X		
Gymnodinium minor	X		
Gymnodinium achromaticum	X		
Gyrodinium fissum	X		
Gyrodinium pingue	X		
Polykrikos barnegatensis	X		
Polykrikos sp.	X		
Phytomonadida			
Pyramimonas reticulata	X		
Pyramimonas sp.	X	X	
Polyoma papillatum	X	X	
Polytomella sp.	X		
Collodictyon triciliatum	X		
Pedimonas minor	X	X	X
Chlamydomonas mikroplankton	X		
Chlamydomonas reinhardii	X		
Chlamydomonas sp.	X		

TABLE I (*Continued*)

	Floating	Attached or in Debris	From Bottom Samples
CLASS			
Mastigophora (cont.)			
SUBCLASS			
Phytomastigoda (cont.)			
ORDER			
Phytomonadida (cont.)			
Sphærella atlantica	X		
Sphærella marina	X		
Chlorogonium euchlora	X	X	
Hyalogonium acus	X	X	
Phacotus sp.	X	X	
Euglenidae			
Euglena gracilis	X		
Euglena sp.	X		
Trachelomonas euchlora	X		
Eutreptia viridis	X	X	
Euglenopsis sp.	X	X	
Astasia lagenula		X	X
Astasia sp.		X	X
Urceolus sabulosus		X	X
Peranema trichophorum		X	X
Petalomonas carinata		X	X
Petalomonas mediocanellata		X	X
Tropidoscyphus octacostatus		X	X
Distigma urceolaris		X	X
Heteronema acutissimum	X	X	X
Heteronema sp.		X	X
Notosolenus orbicularis	X	X	X
Notosolenus apocamptus		X	X
Anisonema ovale		X	X
Anisonema vitrea		X	X
Anisonema truncatum		X	X
Dinema grisoleum		X	X
Chloromonadida			
Thaumatomastix setifera	X	X	X
Gonyostomum depressum	X	X	

	Floating	Attached or in Debris	From Bottom Samples
CLASS			
Mastigophora (cont.)			
SUBCLASS			
Zoomastigoda			
ORDER			
Pantastomatida			
Multicilia lacustris	X	X	
Actinomonas sp.	X	X	
Cercomastix sp.		X	X
Mastigamœba longifilum		X	X
Mastigamœba socialis		X	X
Mastigella mitens		X	X
Bodopsis sp.		X	
Cercobodo crassicauda		X	X
Cercobodo longicauda		X	X
Cercobodo bodo	X	X	X
Cercobodo sp.		X	
Protomastigida			
Dallingeria drysdali	X	X	X
Monosiga ovata		X	
Salpingœca minuta		X	
Salpingœca sp.		X	
Bicocœca oculata		X	
Amphimonas globosa	X	X	X
Monas socialis	X	X	
Monas vivipara		X	X
Monas dangeardii		X	X
Bodo globosus		X	
Bodo lens		X	
Bodo ovatus		X	
Rhynchomonas sp.	X	X	X
Ancyromonas contorta	X	X	
Dinomonas vorax	X	X	
Pleuromonas jaculans		X	X
Oicomonas termo		X	X
Oicomonas socialis		X	
Polymastigida			
Tetramitus sp.	X	X	X
Hexamitus sp.	X	X	X
Trepomonas agilis	X	X	X

TABLE I (*Continued*)

	Floating	Attached or in Debris	From Bottom Samples
CLASS			
Infusoria			
SUBCLASS			
Ciliata			
ORDER			
Holotrichida			
Holophrya discolor		X	X
Trachelocerca papillosa		X	X
Trachelocerca phœnicopterus		X	X
Trachelophyllum sp.		X	X
Lacrymaria lagenula	X	X	X
Lacrymaria olor		X	X
Blepharostoma pigerrima		X	X
Chœnia elongata		X	X
Chœnia teres		X	X
Prorodon teres		X	
Mesodinium rubrum	X	X	
Mesodinium pulex	X	X	
Lionotus cygnus		X	
Lionotus fœciola		X	
Loxodes rostrum		X	
Chlamydodon memosyne		X	X
Chilodon sp.	X	X	
Chilodon cucullulus	X	X	X
Nassula oblonga		X	
Nassula aurea		X	
Dysteria pusilla		X	
Dysteria armata		X	
Trochilia sigmoides		X	X
Ægyria olivia		X	X
Ægyria roseus		X	
Urocentrum turbo	X	X	X
Loxocephalus granulosus	X	X	
Uronema marina	X	X	
Frontonia leucas	X	X	
Glaucoma sp.	X	X	
Colpoda sp.	X	X	
Colpidium sp.	X	X	
Cinetochilum margaritaceum	X	X	X
Paramecium sp.		X	
Pleuronema chrysalis		X	

	Floating	Attached or in Debris	From Bottom Samples
CLASS			
Infusoria (cont.)			
SUBCLASS			
Ciliata (cont.)			
ORDER			
Holotrichida (cont.)			
Cyclidium glaucoma	X	X	X
Lembus pusillus	X	X	
Lembus infusionem	X	X	X
Lembus punctifera		X	
Plagiopyla nasuta		X	
Drepanomonas sp.		X	
Heterotrichida			
Folliculina ampulla		X	
Folliculina elegans		X	
Climacostomum sp.		X	
Condylostoma patens		X	X
Saprodinium sp.	X	X	
Oligotrichida			
Halteria grandinella	X	X	
Labœa spp.	X	X	
Strombidium sulcatum	X	X	
Strombidium grande	X	X	
Strombidium caudatum	X	X	
Tintinnopsis beroidea	X		
Tintinnopsis sp.	X		
Amphorella sp.	X	X	
Hypotrichida			
Peritromus emmæ		X	X
Oxytrichia pelionella		X	
Oxytrichia fallax		X	
Stylonichia mytilus		X	X
Epiclintes radiosa		X	
Epiclintes ambiguus		X	X
Epiclintes retracta		X	X
Amphisia kessleri		X	
Holosticha rubrum	X	X	X
Holosticha pernix		X	
Holosticha multinucleata		X	
Stichotricha secunda		X	X
Histrio pelionella		X	
Uroleptus pisces	X	X	
Uronychia setigera	X	X	
Uronychia transfuga		X	
Euplotes vannus		X	

TABLE I (*Continued*)

	Floating	Attached or in Debris	From Bottom Samples
CLASS			
Infusoria (cont)			
SUBCLASS			
Ciliata (cont.)			
ORDER			
Hypotrichida (cont.)			
Euplotes harpa		X	
Euplotes patella		X	
Diophrys norvegicus		X	X
Diophrys excavatum		X	
Diophrys appendiculatus		X	
Aspidisca hexeris		X	X
Aspidisca costata	X	X	X
Aspidisca turrita		X	
Onychaspis polystyla		X	
Onychaspis leptaspis		X	

	Floating	Attached or in Debris	From Bottom Samples
CLASS			
Infusoria (cont.)			
SUBCLASS			
Ciliata (cont.)			
ORDER			
Peritrichida			
Vorticella spp.		X	
Rhabdostyla spp.		X	
Colhurnia spp.		X	
Zoothamnium spp.		X	
Vaginicola sp.		X	
SUBCLASS			
Suctoria			
Acineta tuberosa		X	
Acineta sp.		X	
Ephelota coronata		X	
Ephelota sp.		X	
Podophrya fixa		X	

By centrifuging known volumes of sea water and carefully decanting, the catch was concentrated for examining living animals. A Spencer binocular microscope with apochromatic equipment and a mechanical stage were used for this. A 60X water immersion objective proved very valuable, but the metallic mounting was quickly toxic to various Protozoa. Because a Sedgwick-Rafter cell did not permit careful examination or counting of small cells, several drops of a well mixed catch delivered by a calibrated pipette were studied for each sample. The only preserved samples studied were those secured on cruises.

Many forms were found which were already familiar as fresh water species, but many others were observed which could not be traced to previously described species. When one of these unfamiliar or apparently undescribed forms appeared in numbers and on more than one occasion an effort was made to study it as a new species.

GENERA AND SPECIES PRESENT

Table I lists 264 genera and species of Protozoa identified from the collections made during the summer of 1934. It will be noted that some groups are very scantily treated. Thus little attempt was made to identify the several species of amœbas which were found, and the few Radiolaria which appeared in the catches about the end of the summer were likewise not identified. No parasitic forms were studied and very little attention was paid to the Suctoria.

The Sarcodina were the least important group in variety and actual numbers found. The Ciliata formed a large and varied group on the bottom and in debris but only a few occurred as free swimming or floating forms. Dinoflagellida and Silicoflagellida were numerous but curiously very few Coccolithophora appeared. Colorless, often large, Euglenida were abundant in samples containing debris and at the bottom. The Pantastomatida were very common. Samples often swarmed with extremely large numbers of small flagellated Protozoa laborious to count and identify which were usually examined to see if they could be identified as *Bodo, Dallingeria* or such small species and were then treated simply as "small flagellates." Altogether it is the Flagellata which are of most importance as swimming and suspended forms.

Of the major groups of Protozoa as listed by Calkins (1926), the Woods Hole waters contain numerous species of each in abundance. This includes the nine free living orders of Mastigophora, and all orders of Infusoria, while the only order of the Sarcodina for which representatives were not identified is the Mycetozoa.

Seasonal Succession

No great pulses of Protozoa were found comparable to the well-known succession of diatoms in the sea. Thus no group of Protozoa ever attained the volume content of *Chætoceros* or *Rhizosolenia* at any observed time in the summer of 1934. These large diatoms more than compensated by their size for any discrepancy in numbers between themselves and the small flagellates, for example.

Practically the only Protozoa showing any succession were the dinoflagellates and silicoflagellates. Radiolaria appeared in small numbers in late August and early September but most of the Sarcodina and Mastigophora and all of the Infusoria were present to some extent at all times. *Distephanus speculum* was the sole representative of its group in early June but later its numbers decreased and other genera and species of silicoflagellates appeared. *Tintinnopsis beroidea* varied greatly in numbers throughout the summer but some were always present. Very few dinoflagellates were present early in the summer but later they became the dominant forms. Thus *Ceratium tripos* was first observed June 24, but soon thereafter it was common, and the same was true of *Peridiniopsis*. The maximum for dinoflagellate species and numbers was about mid-August. No swarming of dinoflagellates occurred either, such as that observed by Martin and Nelson (1929) in Delaware Bay. Table II gives the numbers and seasonal occurrence of various groupings of Protozoa at Woods Hole in the summer of 1934.

TABLE II

Estimated numbers of Protozoa per liter of sea water showing seasonal occurrence for each of four species, five related groups and totals of all Protozoa occurring at a given sampling point in the Hole at Woods Hole in the summer of 1934.

Date	*Exuviaella lima*	*Prorocentrum micans*	*Distephanus speculum*	*Tintinnopsis beroidea*	Heliozoa	Euglenoid Flagellida	Total Dino-Flagellida	Total Flagellida	Total Ciliata	Total Protozoa
June 14...	1,500	375	7,875	1,500	—	—	13,875	25,500	1,875	27,375
" 16...	312	—	3,750	1,662	—	—	7,187	27,874	2,599	30,500
" 19...	1,270	—	3,810	—	—	—	5,715	115,555	300	115,855
" 21...	450	—	6,750	1,575	—	225	9,900	29,025	1,800	33,300
" 22...	1,575	—	4,725	5,250	—	—	8,400	27,825	5,250	33,075
" 23...	2,520	840	1,680	3,360	—	—	11,660	28,206	3,570	31,750
" 24...	5,940	—	3,780	1,080	—	—	13,500	58,320	1,620	59,940
" 27...	3,500	—	3,000	1,000	—	—	5,000	52,500	1,500	54,000
July 3...	5,500	4,000	8,500	8,500	—	3,000	23,000	53,750	12,500	69,250
" 10...	8,500	1,000	3,500	7,000	3,000	2,500	28,500	83,000	9,500	95,900
" 18...	6,500	2,750	2,750	3,250	3,250	3,250	15,500	90,250	4,750	100,500
" 31...	6,750	6,375	1,500	9,375	—	300	36,275	93,500	31,875	125,375
Aug. 8...	1,500	—	500	2,625	—	750	16,875	68,250	8,250	76,490
" 15...	5,250	5,625	—	9,750	1,500	4,175	46,875	178,125	15,750	135,375
" 22...	7,875	3,375	—	6,375	1,875	3,750	31,875	111,720	9,375	122,970
" 29...	6,750	5,250	—	7,500	2,250	1,500	40,875	102,395	13,875	119,270

NATURE AND ACCUMULATION OF SEA FLOOR POPULATION

The rate at which the protistan flora and fauna become established on the bottom of the sea in summer, at least in shallow waters, is amazing. Syracuse dishes were suspended from the float enclosed in wire cages so that only very small organisms could have access to them. At stated periods a dish was removed, wiped clean underneath, and its contents examined with a water immersion objective. It was soon found that direct examination was no longer possible after 24 hours; sediment and organisms were too thick to see through after that interval. Table III gives the numbers of identified organisms which accumulated in a representative dish in 24 hours between July 9 and 10.

It will be seen from Table III that the population consisted of about one-third each of Protozoa, diatoms, algæ and fungi. In this average sample the number of organisms listed is smaller than the number actually accumulating in 24 hours. Some are out of the plane of observation, some are obscured by debris, some encyst in the allotted period, and some occur which cannot be identified. Small samples of film from such a Syracuse dish were examined and the only organisms which could be found were diatoms, amœbæ, the ciliates *Cyclidium* and

Lembus and the flagellates *Bodo* and *Rhynchomonas.* These samples were then added to filtered sea water enriched with nutrient materials and gave thriving cultures of several genera of diatoms; four species of amœbas; the ciliates *Uronychia, Cyclidium, Lembus, Holosticha, Euplotes charon* and *Euplotes patella* as well as some unidentified ciliates; *Oxyrrhis marina, Amphidinium operculatum* and another unidentified dinoflagellate, *Rhynchomonas, Bodo* and *Notosolenus;* and green, brown, red and blue-green algæ, these last being especially rich in number of species and abundance. Evidently the sea bottom shelters many organisms other than those in the trophic state, and to arrive at the true population one should augment visual examination of fresh samples with

TABLE III

Numbers of protistan organisms accumulating in 24 hours per square centimeter of exposed Syracuse dish. A typical sample.

Diatoms	16,120
Small algal cells	17,980
Fungi	2,480
Unidentified amœbeæ	2,400
Unidentified Heliozoa	620
Unidentified flagellates	5,580
Unidentified ciliates	310
Exuviælla lima	930
Gymnodinium sp.	620
Ceratium lineatus	240
Pyramimonas inconstans	1,240
Calycomonas sp.	2,480
Trachelomonas euchlora	240
Anisonema ovale	620
Notosolenus orbicularis	240
Chlamydodon memosyne	240
Cyclidium glaucoma	240
Trachelocerca phœnicopterus	240
Euplotes patella	240
Aspidisca costata	240
Podophrya fixa	240
Total Protozoa per sq. cm.	17,060

a variety of cultures designed to produce excystment and growth; the cultures above were produced on only one type of medium. Instead of accumulating a population of 144,000 Protozoa per square meter in 24 hours, the sea bottom at this shallow point might acquire many times that many.

The nature of the sea bottom largely determines the nature and rate of accumulation of its population. Sandy or current-swept bottoms might have a small population, except in crevices, depressions or other sheltered situations. In the Syracuse dishes representing a sheltered bottom a film was gradually built up during four weeks greatly reminiscent of

the film accumulating on the stones of the trickling filter in a sewage disposal plant. It consisted of much debris but nematodes, hydroids, and other Metazoa were abundant in it. Its chief population, however, consisted of Protozoa and diatoms with a few filamentous algæ. The deeper parts of the film contained few Protozoa, the maximum population being in the porous upper part. Wherever such a thin blanket of film as this exists on the sea floor it must be of great importance in the mineralization of organic debris, which obviously occurs to the greatest extent only where such a film can be built up. The few samples obtained in 1934 from the sandy bottom of Vineyard Sound yielded few Protozoa, but samples from the mud banks in Great Harbor showed an

TABLE IV

Protozoa in the surface film of two samples from muddy bottoms, and those obtained by towing five feet off bottom where one of the mud samples was obtained.

Buzzards Bay	Great Harbor
Forty-five foot depth	Nine-foot depth
Amœbeæ, three species	Amœbeæ, two species
Foraminifera, four species	Foraminifera, two species
Gromia lagenoides	*Shepheardiella tæniformis*
Anisonema vitrea	*Anisonema vitrea*
Dinema griseolum	*Anisonema* sp.
Distigma proteus	*Heteronema* sp.
Urceolus sabulosus	*Peranema trichophorum*
Monas amœbina	*Urceolus sabulosus*
Monas obliqua	*Mastigamœba longifilum*
Bodo globosus	*Monas vivipara*
Lionotus wrzesniowski	*Bodo angustus*
Loxodes rostrum	*Polytoma uvella*
Peritromus emmæ	*Prorocentrum triangulatum*
Trachelocerca phœnicopterus	*Rhodomonas baltica*
Oxytrichia fallax	*Cyclidium glaucoma*
Uroleptus pisces	*Prorodon* sp.
Diophrys excavatus	*Chlamydodon memosyne*
Tintinnopsis beroidea	*Trachelocerca phœnicopterus*
Unidentified ciliates, three species	Unidentified ciliate, one species
Towing. Buzzards Bay	
Distephanus crux	*Ochromonas marina*
Exuviella lima	*Exuviella marina*
Ceratium fusus	*Ceratium lineatus*
Gonyaulax triacantha	*Peridinium divergens*
Peridiniopsis asymmetrica	*Phalacroma roseum*
Monas vivipara	*Holophrya griseum*
Strombidium sulcatum	*Tintinnopsis beroidea*

abundance of flagellates and ciliates. Table IV shows the Protozoa identified from two such bottom samples, together with those obtained

from a towing just off the bottom in the same locality for comparison. Bottom-dwellers are largely holozoic or saprozoic, whereas those from towings in shallow water are largely holophytic or saprozoic. The small number of species from the bottom samples is accounted for by the fact that the core of mud in each case was about 18 mm. in diameter. No fresh cores from deep samples were examined in 1934, although a core four days old from a depth of 200 meters contained numerous *Bodo* and *Anisonema* and a new species of *Prorodon*.

The Role of the Protozoa

It seems generally agreed that the Protozoa are important as a food source for other organisms in the sea, especially such animals as copepods. Examination of the gut contents of salps and copepods generally shows an abundance of diatom and dinoflagellate shells as well as those of Tintinnoidea. But such examinations reveal nothing about the use of naked dinoflagellates, and the smaller ciliates and flagellates; they disintegrate and leave no recognizable remains in the gut. I am of the opinion that these two latter types are more important in shallow waters than the shelled forms because of their abundance, and also because the large diatoms like *Chætoceros* and *Rhizosolenia*, which were exceptionally numerous at Woods Hole in 1934, cannot be ingested by the smaller copepods. Direct observation is not very practical, but small copepods were seen feeding on the bacteria and Protozoa in laboratory cultures in July and six months later were abundant in those cultures containing few diatoms but abundant bacteria and Protozoa. Clarke (1935) has proved that copepods use these minute cells as food, and from Table II it may be inferred that they are sufficiently numerous at all times in the summer to serve as food. If these very small protozoan cells are considered to be roughly spherical, an individual 5 microns in diameter would have a volume of 65.45 cubic microns, and a liter could contain roughly 15^{12} such individuals, so that a population of 300,000 per liter which has been noted at Woods Hole would still be a relatively sparse one. Nevertheless, the feeding mechanism of such animals as salps and copepods is perhaps capable of straining out these food organisms and the rapidity of cell division of these small forms permits a speedy recovery.

Nelson (1935) cites the importance of the dinoflagellates as food for oysters and has successfully experimented with the feeding of them to oysters. In areas such as the New Jersey coastal waters where the dinoflagellate population is great they may have a decided importance for this reason. Undoubtedly more careful investigation of the food of

many marine animals will reveal a larger rôle of the Protozoa as food organisms.

The Protozoa also have an important function in the turnover of organic matter; this is not separable from their function as food-forming organisms. Dr. C. J. Fish remarked to me, "There must be numerous Protozoa in the surface waters; if a dead copepod is taken while over Georges Bank and allowed to stand in sea water from the same source, it is quickly attacked by a swarm of Protozoa." This is an easily made observation. Bacteria swarm about the dead animal also and they and the Protozoa penetrate inside the exoskeleton where they are not swept away by currents; there they work rapidly and soon only the chitinous shell remains. Often the visible Protozoa are so thick inside that their volume must be many times that of the decay bacteria. Direct observation is inadequate as proof that they actually ingest the flesh of the dead animal, but a *Loxophyllum* quickly becomes filled with round bodies, presumably stored food, while a *Lembus infusionem* can be seen forming food vacuoles containing ingested bacteria. The larger ciliates may feed on bacteria or on diatoms or other Protozoa and are themselves sizeable food morsels, as a species of *Chænia* and one of *Trachelocerca* each over 1,500 microns long. The holozoic and saprozoic forms attack organic debris in suspension or on the ocean floor and constantly convert it to protoplasm in part and accomplish its mineralization in part. If they do not compare with the bacteria in numbers, their size is a compensation for this.

Some Protozoa, especially the smaller ones, may be a part of the food chain by partly acting as "food condensers." Butterfield (1933) has shown that some bacteria thrive in a food medium too dilute for the ciliate *Colpidium*, which alone requires 500 mg. per liter of certain substances, but which will grow on 5 mg. per liter of these substances if *Bacillus ærogenes* is present, the bacterium apparently "condensing" the dilute food substances and being eaten in turn by the ciliate. It seems probable that the numerous smaller and presumably saprozoic flagellates may condense food in the sea in a similar manner, and in turn become food for larger forms.

The photosynthetic activities of the Protozoa are also important. The pigmented forms are numerous, and have a separate value in the food chain because of their utilization of inorganic matter. This work of those containing chlorophyll is self evident, but many brown, red and other coloring matters are found in the various groups, and the functions of these coloring matters is unknown. In the Woods Hole area pigmented Rhizopoda are principally Heliozoa and few in number, but pelagic Radiolaria containing the yellow symbiotic flagellate, *Chrysi-*

della, are abundant in the open sea. Among the flagellates four of the orders contain many pigmented forms, the most important being the Dinoflagellida. Chlorophyll is practically lacking in the marine Infusoria but other colors are not.

The elaboration of glucose and subsequent products by these pigmented forms must be evaluated in any consideration of the carbon content of the sea. Furthermore, the depths at which the pigment-bearers are found must be investigated, especially since they are active and rapid swimmers and are more independent of flotation mechanisms than the diatoms.

Conclusions

Systematic examination of the waters in the Woods Hole area has shown the existence of a large protozoan population from early June to mid-September. The population of this shallow tide-swept area is probably greater than hitherto suspected and must have a considerable ecologic importance because of its numbers, ability to reproduce rapidly, place in the food cycle, and furtherance of mineralization of organic debris. Scant examination of the off-shore waters indicates that the large inshore numbers are approximated in these off-shore waters also. Chlorophyll and other pigment-bearing forms predominate near the surface while at the bottom bacterial feeders and saprozoic forms are most abundant. Changes in the dinoflagellate population suggest seasonal succession but no such changes were observed among other types. The fauna at the bottom accumulates rapidly when protected and its members are active in the constant mineralization of the deposited debris. The maximum numbers in this film are determined by its thickness and rate of deposition. A weekly census of organisms present was kept, and a check list of the genera and species observed in the area has been made.

BIBLIOGRAPHY

Allen, E. J., 1919. A contribution to the quantitative study of plankton. *Jour. Mar. Biol. Assn'n, N. S.*, **12**: 1.

Brady, H. B., 1884. Report on the Foraminifera. In "The Voyage of H. M. S. Challenger," Vol. 9.

Butterfield, C., 1933. A report on the food habits of Colpidium. *U. S. Pub. Health Reports*, **48**: 28.

Calkins, G. N., 1902. Marine Protozoa from Woods Hole. *Bull. U. S. Fish. Comm.*, **21**: 413.

Calkins, G. N., 1926. Biology of the Protozoa. Lea and Febiger, Philadelphia.

Clarke, G. L., and S. S. Gellis, 1935. The nutrition of copepods in relation to the food-cycle of the sea. *Biol. Bull.*, **68**: 231.

Clemens, W. A., 1933. A Check-List of the Marine Fauna and Flora of the Canadian Pacific Coast. National Research Council of Canada. Ottowa.

Cleve, P. T., 1899. Plankton researches in 1897. *Kong. Svensk. Vetensk.-Akad. Handl.*, vol. 32.

CLEVE, P. T., 1901. The plankton of the North Sea, the English Channel, and the Skagerack in 1899. *Kong. Svensk.-Akad. Handl.*, vol. 34.

CUSHMAN, JOSEPH A., 1908. Foraminifera of the Woods Hole Region. *Proc. Boston Soc. Nat. Hist.*, **34**: 22.

FAURÉ-FRÉMIET, E., 1924. Contribution a la Connaissance des Infusoires Planktoniques. *Bull. Biol. France*, Belg. Supp. 6.

GRAN, H. H., 1912. The plankton production of the North European waters in the spring of 1912. *Bull. Planktonique.*

GRAN, H. H., 1912. Pelagic Plant Life. In "Depths of the Ocean," by Sir John Murray and Dr. Johan Hjort. MacMillan and Co., London.

HERDMAN, W. A., AND A. SCOTT, 1908–1915. An Intensive Study of the Marine Plankton around the South End of the Isle of Man. Parts 1–8, Reports for 1907–14 of the Lancashire Fish. Lab.

JOHNSTONE, JAMES, A. SCOTT, AND H. C. CHADWICK, 1924. The Marine Plankton. The University Press of Liverpool.

JORGENSEN, E., 1927. Tintinnidæ. In "Die Tierwelt der Nord- und Ostsee." Lief VIII, Teil. II.

KAHL, A., 1928. Die Infusorien (Ciliata) der Oldsloer Salzwasserstellen. *Arch. f. Hydrobiol.*, vol. 19.

KOFOID, C. A., AND OLIVE SWEZY, 1921. The free-living unarmored dinoflagellata. *Mem. Univ. Calif.*, Vol. 5.

LEBOUR, MARIE V., 1917. The microplankton of Plymouth Sound from the region beyond the breakwater. *Jour. Mar. Biol. Ass'n*, **11**: 133.

LEBOUR, MARIE V., 1925. The Dinoflagellates of Northern Seas. William Brendon and Sons, Plymouth.

LOHMANN, H., 1902. Die Coccolithophoridæ. *Arch. f. Protist.*, **1**: 1.

MARTIN, G. W., 1929. Dinoflagellates from marine and brackish waters of New Jersey. *Univ. Iowa Studies*, **12**: 9.

MARTIN, G. W., AND THURLOW C. NELSON, 1929. Swarming of dinoflagellates in Delaware Bay, New Jersey. *Bot. Gaz.*, **88**: 2.

NELSON, THURLOW C., 1935. Personal communication to the author.

RHUMBLER, L., 1900. Nordische Plankton. Foraminiferen.

SCHAEFFER, A. A., 1923. On the distribution of marine amebas. *Carneg. Inst. Wash. Year Book* No. 21, for 1922, p. 177.

SCHAEFFER, A. A., 1924. Investigations on marine amœbas. *Carneg. Inst. Wash. Year Book* No. 23, p. 199.

SCHAEFFER, A. A., 1926*a*. Investigations on marine amebas in Newfoundland and Labrador. *Carneg. Inst. Wash. Year Book* No. 25, p. 312.

SCHAEFFER, A. A., 1926*b*. Taxonomy of the amœbas. *Carneg. Inst. Wash., Publ.* No. 345.

SCHILLER, JOSEPH, 1926. Die planktonischen Vegetationen des adriatischen Meeres. B. Chrysomonadinæ, Heterokontæ, Cryptomonadinæ, Eugleninæ, Volvocales. *Arch. f. Protist.*, **53**: 59.

SMITH, J. S., 1904. A Preliminary Contribution to the Protozoan Fauna of the Gulf Biologic Station, with Notes on Some Rare Species. Bull. 2, Second Report, Gulf Biologic Station.

EFFECT OF ULTRAVIOLET RADIATION ON THE HEART OF LIMULUS POLYPHEMUS

S. A. GUTTMAN

(From the Department of Physiology and Biochemistry, Cornell University Medical College, Ithaca, N. Y., and Marine Biological Laboratory, Woods Hole, Mass.)

The heart of *Limulus polyphemus* was excised along with the dorsal ganglion. The rhythmically beating heart was then placed in a finger bowl containing 200 cc. of sea water. Temperature changes were observed by means of a thermojunction. One blackened junction was placed on the ganglion at the level of the sixth segment. As the heartbeat of the *Limulus* is neurogenic (Carlson, 1904 and 1905, and Garrey, 1932), its frequency and amplitude depend on the action of the ganglion. Thus, if the temperature of the ganglion is recorded it is easy to determine whether frequency changes are attributable to temperature variations of the pace-maker. The source of ultraviolet employed during this investigation was a Cooper-Hewitt Uviarc, with a 6-inch tube and reflector, placed at 35 cm. from the preparation.

Figure 1 indicates the effects of repeated short intense ultraviolet radiations on the *Limulus* heart with the dorsal or ganglion surface receiving the radiation. The temperature of the ganglion is also graphed (broken line curve). It is quite apparent that the temperature increases caused by the heat of the mercury arc were not responsible for the observed changes as the increases in temprature never exceeded 0.3° C. during a 3-minute period of radiation. Furthermore, while there was a progressive rise in temperature, it was slight and was not accompanied by a corresponding progressive and sustained increase in cardiac rate; on the contrary, each brief exposure of the heart to the action of ultraviolet light was accompanied by a burst of acceleration which was followed in each instance by a recession of rhythm to the initial rate or even to a slower rhythm when the exposure ceased. The general tendency of the temperature curve is toward a higher level. This is attributable in the main to the fact that the room temperature was higher than the initial temperature of the bath although the radiation may have added, in a minor degree, to the rise of temperature of the bath.

It was observed that the amplitude of the heart's contractions also

increased when these short doses were applied. No correlation between increase in frequency and in amplitude was found.

Shielding the heart with a glass plate and radiating the heart had no effect. Thus, it is concluded that rays longer than 3,500 Angstrom units are not responsible for the results reported and that it is the ultraviolet region which is the factor to be considered.

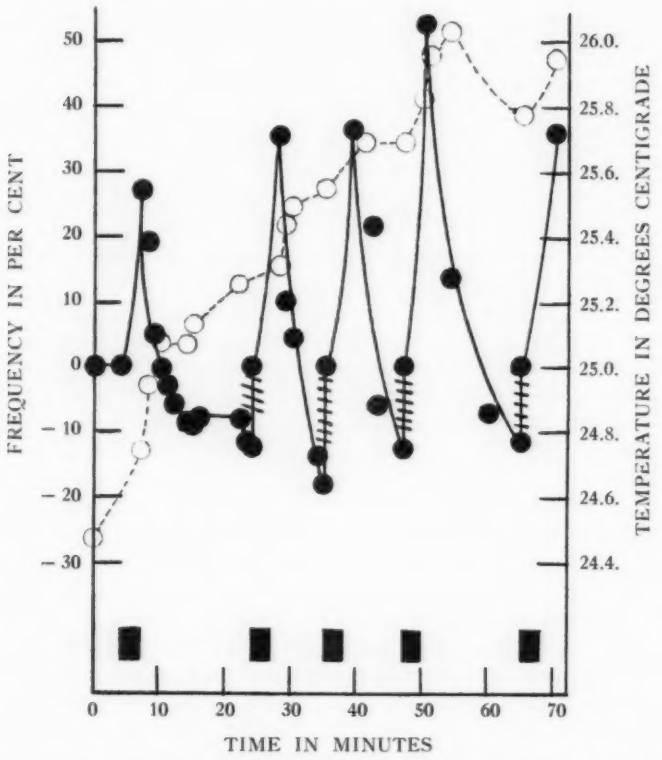


FIG. 1. Graph indicating the effect of ultraviolet light on frequency of *Limulus* heart-beat. ● frequency; ○ temperature; ■ period of radiation.

DISCUSSION

The *Limulus* heart responds to ultraviolet radiation in a manner similar to that of the frog and alligator (Guttman, 1935). Since the *Limulus* heart-beat is neurogenic, the above experiments demonstrate

the definite stimulating effect of ultraviolet light of rhythmogenic processes in nerve cells of the cardiac ganglion.

It was observed that by the end of 3 minutes of radiation the maximum increase appeared and then a rapid fall of the indices ensued. Radiation for longer periods caused a greater fall in the frequency and amplitude values after the initial increase. Hinrichs (1926) states that "the amount of radiation in a given region of the spectrum, which is absorbed by the protoplasm in a given period of time, determines whether there is to be an increase or decrease in the speed of the physiological process." Packard (1931) working with X-ray, reports "an acceleration which is followed by a retardation and often by a very obvious injury."

It may be supposed, in the case of the *Limulus* heart, that the primary action of the radiation is to increase the frequency of the nerve cell discharges and also to increase the number of active nerve cells, hence the number of nerve fibers transmitting impulses to the myocardium is increased. The former supposition would account for the increased frequency, the latter for the increased amplitude. The secondary effects (the decrease in the indices) may be attributed to a decrease in the number of fibers transmitting impulses and a decrease in their frequency.

I am greatly indebted to Professor W. E. Garrey of Vanderbilt University Medical College, Nashville, Tennessee, for his generous advice and aid.

LITERATURE CITED

Carlson, A. J., 1904. *Am. Jour. Physiol.*, **12**: 67.
Carlson, A. J., 1905. *Ibid.*, **12**: 471.
Garrey, W. E., 1932. *Collecting Net*, **7**: 137.
Guttman, S. A., 1935. *Proc. Soc. Exper. Biol. and Med.*, **33**: 408.
Hinrichs, M. A., 1928. *Proc. Soc. Exper. Biol. and Med.*, **26**: 175.
Packard, C., 1931. *Quart. Rev. Biol.* **6**: 253.

OXYGEN PRODUCTION BY ZOÖXANTHELLÆ IN A BERMUDAN TURBELLARIAN

MARTHA F. WELSH

(From the Bermuda Biological Station for Research)

An intimate association of plants and animals such as the occurrence of Zoöchlorellæ and Zoöxanthellæ in certain protozoans, cœlenterates, and flatworms presents some interesting problems which have long attracted biologists. The most extensive studies have been made by Keeble and Gamble on the two species of *Convoluta* found at Roscoff. Many interesting observations were made by these investigators but one of the most important, the production of oxygen by the algæ as a result of photosynthesis and the subsequent use of this oxygen by the animal, was not extensively studied. It has been easier in the case of the green planarian, *Convoluta roscoffensis*, than in the case of the yellow form, *C. paradoxa*, to demonstrate that photosynthesis occurs and that the animal benefits from the presence of the algæ in its body. Geddes (1879) and Haberlandt (1891) found the green planarian when placed in darkness dies in the course of two or three days, the implication being that when photosynthesis is arrested death from starvation ensues. Geddes with a simple experiment, testing by a glowing match tip, was the first to show that the gas given off in the sunshine by this same form was oxygen. Keeble (1910), in summing up the work carried on jointly with Gamble, says that the green cells in *C. roscoffensis* are capable of photosynthesis and, when exposed to light, can utilize carbon dioxide, give off oxygen, and manufacture carbohydrates the excess of which is stored as starch. This starch in turn is used by the animal, as is shown by its disappearance in animals kept in the dark. Keeble (1908, 1910) said it was not easy to prove that the yellow-brown cells of *C. paradoxa* were capable of photosynthesis but finally concluded that the reserve food contained in these algal cells was probably a product of photosynthesis, and served the animal for nutritive purposes. He claims the reserve food is a fatty substance, in the form of droplets and is not starch, thus differing from the stored food of the green form. Since *C. roscoffensis* and *C. paradoxa* have no excretory system, Keeble states the algal cells in both forms act as excretory organs and use the waste nitrogen products of the animal.

The object of the present investigation was to determine whether a

sufficient amount of oxygen might be produced by Zoöxanthellæ to be of value to flatworms harboring them and if so, to determine the quantitative relations between oxygen production and consumption in the light and in the dark. A form suitable for this work was found in abundance during the summer months, in the aquaria at the Bermuda Biological Station, where this investigation was carried on during June and July, 1933 and 1935. This turbellarian is probably *Amphiscolops langerhansii,* described by von Graff (1905), but the description by Haswell (1905) of an Australian acœlous turbellarian *Heterochærus* is in some respects a better description of the Bermuda form, particularly of the reproductive apparatus. However, it is believed that the two forms are identical and the genus *Amphiscolops* is already well established. *Amphiscolops langerhansii* according to von Graff occurs in nature in shallow rock-pools between tidal limits on the brown seaweed and sand, but several attempts failed to locate such a source and the laboratory aquaria supplied the several hundred specimens used in the course of the work.

These turbellarians are positively phototropic and gather on the sides of the aquaria toward the light. They are bilobed at the posterior end and when not stretched out in moving, measure 2 to 3 mm. long and 1 to 1.3 mm. wide. There are two red eye spots and a statocyst easily seen in animals which have been kept in the dark and whose color is lighter due to fading and shrinking of the Zoöxanthellæ. Besides the brown color due to the many Zoöxanthellæ, there is a reflecting pigment scattered over the dorsal surface in a more or less median line.

The Zoöxanthellæ are not seen in a newly hatched larva, but appear after a few days and in the adult measure 0.020 to 0.022 mm. in diameter. These algal cells in a healthy adult worm, when stained with iodine, are seen to contain small bluish bodies which are evidently starch. When treated with 95 per cent alcohol the Zoöxanthellæ lose the yellow-brown color first, then the chlorophyll collects as light green droplets and begins to exude from the algæ as their cell walls break down and they disintegrate.

The worms were collected from the sides of the aquaria by means of a pipette and placed in 70 cc. bottles filled with sea water which had been allowed to stand for at least an hour in order to permit the escape of excess oxygen. The number of animals per bottle varied from one to thirty. The ground-glass stoppers were water-sealed in order to prevent an exchange of gases with the air, and the bottles were placed either in a dark-room or in a situation where they received bright diffuse light during daylight hours. The object was to determine whether *Amphiscolops* kept in the light would survive longer than when kept in

the dark and also whether a few animals would survive longer than many animals in a limited amount of oxygen. The bottles were examined at least once each day and the number of days the worms survived was noted. All of the individuals of a given bottle died within a few hours of one another, thus making it easy to determine the number of days of survival of the group. It is possible that the first dying worms had a toxic effect on the remainder and this might account for the individuals of a given group dying so nearly simultaneously. However, the uniformly consistent results obtained in all experiments indicated that the chance death of an animal, and its possible toxic effect on the remainder could be of little significance. The results are shown in Table I. In the tests carried on in the dark it may be seen that in all cases

TABLE I

Showing time of survival of worms in sealed bottles kept in constant darkness, except during periods of examination.

No. of worms	1	5	10	20	30
No. of days survival....	20+	6	4	3	2
	13+	8	6	3	2
	13+	5	3	3	2
	13+	7	3		3
	16+	4	5		
		6	3		
Averages.....	15+	6	4	3	2.2

Showing time of survival of controls in diffuse light, for experiments carried on during June and July, 1935.

No. of worms	1	5	10	20	30
No. of days.....	19+	19+	19+	19+	16+

single individuals were surviving when the experiment had to be discontinued, giving an average survival time of more than fifteen days. With five animals per bottle the time of survival was reduced to six days on the average, and a gradual reduction may be noted until for thirty animals the time was 2.2 days. Only one series of controls in the light is shown, that done in 1935. They were not all started on the same date but all were surviving at the end of sixteen or nineteen days, which was as long as it was possible to make observations. It is evident from these tests that some product of the photosynthesis carried

on by the symbiotic algæ benefits the animals which harbor the algæ. To what extent it may be the oxygen produced will be seen in the experiments which are now to be discussed.

Sealed bottles containing one to thirty worms in sea water were prepared as in the survival tests and one of a pair placed in the dark, the other in bright diffuse daylight. While no attempt was made to control the temperature, it fluctuated only slightly about an average of 26° C. After the bottles had remained in the light or in the dark for

TABLE II

Showing the amounts of oxygen in bottles containing one to thirty worms kept for a period of days in the light and in the dark.

No. of worms	Day tested	Dark	Light
		cc./liter	*cc./liter*
1	4	3.82	4.72
1	4	4.22	3.37
1	5	3.13	4.03
1	5	3.13	
Averages	4.5	3.58	4.04
5	4	3.26	4.38
5	4	3.37	4.61
Averages	4	3.31	4.50
10	4	2.52	4.57
10	4	1.49	4.57
10	4	2.06	4.80
10	4	1.79	4.14
10	4	2.24	
10	4	2.01	4.47
Averages	4	2.02	4.51
20	2.5	1.37	4.57
Averages	2.5	1.37	4.57
30	2	.23	4.05
30	2	.67	
Averages	2	.45	4.05

two to five days and before any of the worms had begun to die, oxygen tests were made. The Winkler method according to Kemmerer, Bovard, and Boorman (1923) was used. The results may be seen in Table II. The amount of dissolved oxygen in the sea water at the beginning of

the experiments ranged from 4.0 to 4.5 cc. per liter. The oxygen content of the sea water was not determined for each set of experiments. The sea water used in each of the bottles of a given pair came from the same sample, however, and the light series serves as a control, as the object was to determine the oxygen consumption in the dark. Table II gives the amounts at the end of two to five days for varying numbers of worms kept in the light and in the dark. In the light the average amounts of oxygen may be seen to vary from 4.04 to 4.57 cc. per liter, showing that under normal light conditions enough oxygen is produced by photosynthesis to supply the needs of the worms and to maintain a comparatively constant amount of dissolved oxygen.

If we now examine the results of the tests made on animals kept in the dark it may be seen that in the case of one animal the average

TABLE III

Showing the difference in oxygen content of the light and dark series of Table II, which gives a relative measure of oxygen consumption in the dark. From this it is possible to calculate the oxygen consumed by one worm in a day. It is necessary to assume that in the light the oxygen content is maintained at approximately its original level by the photosynthetic activity of the algæ.

No. worms	Day tested	Total O_2 consumed	O_2/day	O_2/worm/day
		cc./liter	*cc.*	*cc.*
1	4.5	0.46	0.10	0.10
5	4	1.19	0.30	0.059
10	4	2.49	0.62	0.062
20	2.5	3.20	1.28	0.064
30	2	3.60	1.80	0.060
			Average 0.069 cc./liter	

volume of oxygen remaining at the end of four and one-half days was 3.58 cc. per liter and a gradual reduction in oxygen occurred with an increase in the number of animals per bottle, until for thirty animals only 0.45 cc. per liter remained at the end of two days. These results indicate that in the dark a group of *Amphiscolops* soon uses the limited supply of oxygen and the Zoöxanthellæ are naturally unable to replace it. In fact, in the absence of light the symbiotic algæ must be a distinct burden to the worms for they likewise utilize some oxygen and also fail to remove the carbon dioxide and other waste products of the worms.

From the data of Table II it is possible to calculate the rate of oxygen consumption in the dark and this is shown in Table III. Except in the case of single worms the amount of oxygen consumed per worm

per day does not vary much from the average of 0.069 cc. per liter per day. This indicates that the method employed and the results obtained are quite reliable.

In view of the fact that in daylight the dissolved oxygen is maintained approximately constant, this average of 0.069 cc. per liter also represents roughly the amount of oxygen produced per day by the Zooxanthellæ of a single worm. The symbiotic algæ of *Amphiscolops*, although yellow-brown algæ, are therefore seen to produce enough oxygen by photosynthesis to supply the needs of the worms which harbor them; thus they are just as valuable as are the Zoöchlorellæ of *Convoluta*.

LITERATURE CITED

GEDDES, P., 1879. Observations on the physiology and histology of Convoluta Schultzii. *Proc. Roy. Soc. London*, **28:** 449.

HABERLANDT, G., 1891. Uber den Bau u. d. Bedeutung der Chlorophyllzellen von Convoluta roscoffensis. Appendix to von Graff's work, p. 76. Von Graff, Die Organisation der Turbellaria Acœla. Leipzig, Engelmann, 1891.

HASWELL, W. A., 1905. Studies on the Turbellaria. *Quart. Jour. Micr. Sci.*, **49:** 425.

KEEBLE, F., 1908. The yellow-brown cells of Convoluta paradoxa. *Quart. Jour. Micr. Sci.*, **52:** 431.

KEEBLE, F., 1910. Plant-Animals. Cambridge.

KEMMERER, G., J. F. BOVARD, AND W. R. BOORMAN. 1923. Northwestern lakes of the United States. Biological and chemical studies with reference to possibilities in production of fish. *Bull. U. S. Bur. Fish.*, **39:** 51.

VON GRAFF, L., 1905. Das Tierreich. Lief 23. Turbellaria I. Acœla. Berlin.

A CHROMOSOME STUDY OF THE APHID MACROSIPHUM SOLANIFOLII

CHESTER A. LAWSON

(From the Department of Zoölogy, University of Michigan)

Introduction

The extensive genetic work being done in this laboratory on the cycle of *Macrosiphum solanifolii* with respect to wing production, the gamic forms, and intermediates should eventually necessitate a study of the possible chromosome relations with the cyclical phenomena. The present work, dealing primarily with the normal chromosome behavior, will serve as a basis for such an investigation. After the problem had been under investigation for some time cytological results on other species of aphids (Jeffrey, 1933, and Suomalainen, 1933) were seen to be not in agreement with previous work done mainly by Morgan (1908, 1909, 1910, 1912, 1915) and von Baehr (1908, 1909). There was thus a second object in the present study—that of throwing light on the disagreement between the new work and the old.

The problem was suggested by Professor A. Franklin Shull and throughout its progress his generous helpfulness and pertinent suggestions have been of inestimable value.

Life Cycle

Parthenogenetic wingless females of *Macrosiphum solanifolii* appear on the rose plant in the spring, presumably developing from fertilized eggs laid the preceding fall. These so-called stem mothers give birth parthenogenetically to winged or wingless parthenogenetic females. Succeeding generations are likewise both winged and wingless in variable proportions. The potato serves as the summer host. Migration to it occurs in several generations or possibly in all generations as long as any aphids are left on the rose, and some individuals may remain on the rose throughout the summer.

During the summer these two types, the winged and wingless parthenogenetic females, continue to bring forth successive generations of parthenogenetic young, the winged forms producing more wingless offspring than the wingless ones produce.

In the fall the winged forms give birth to gamic females. This may

occur on the rose if the winged mother of the gamic female has migrated thither. The males, which are winged and are born mostly of wingless mothers, develop on the potato plant and are believed to seek out the females on the rose. After fertilization the eggs are laid. These eggs overwinter presumably on the rose, and from them stem mothers develop in the spring.

Material and Technique

Unlimited numbers of parthenogenetic females of the potato aphid were easily maintained throughout the entire year on potato plants grown in flower pots in the laboratory. The males occurred in smaller numbers and at long intervals so that whenever they did appear it was necessary to preserve for future study as many as could be handled.

For a study of the maturation stages of the parthenogenetically developing egg, the adult parthenogenetic wingless females were fixed and sectioned, and studies were made of the ovarioles within the enclosed embryos. The maturation stages in the males were most numerous in the second and third instars.

Navashin's fixative (4 to 12 hours) gave the best results on both chromatin and cytoplasm and was used almost exclusively. The N-butyl alcohol technique for animal tissues described by Stiles (1934) was used for dehydration and infiltration. The insects were embedded in paraffin; sections were cut at 7 micra and stained in Heidenhain's iron hematoxylin.

The drawings of cell outlines were made with the aid of a camera lucida and a Zeiss 2 mm. (1.3) apochromatic objective and a 15 × ocular. These outlines were later enlarged with an opaque projector and details of cells drawn free-hand.

Spermatogenesis

Spermatogonial Mitosis

The testes are bilobed structures composed of a number of cysts containing germ cells in various stages of development. The germ cells in any particular cyst are in the same stage of development and in the embryos these cells are all spermatogonia, many of them undergoing mitotic division.

The resting spermatogonia (Fig. 1) have a characteristic large, rounded nucleus with a heavily staining nucleolus, which in the majority of cases lies near the nuclear membrane. The cytoplasm surrounding the nucleus is a comparatively thin layer and contributes little to the size of the cell.

The chromatin first appears in the early prophase as a network of fine, lightly staining threads connecting heavily staining granules scattered throughout the nucleus. The threads appear single, though it is impossible to determine this with certainty. As the chromatin threads condense they become grouped about the nucleolus, radiating in long loops from this point. The completion of the condensation in late prophase reveals nine chromosomes (Fig. 2), all of which are rod-shaped and of the same thickness, but of five different lengths. The longest chromosome is unpaired. The rest are paired; the longest of the four is slightly shorter than the single chromosome, the smallest is nearly spherical, and the others are of intermediate lengths. The nucleolus is still present at this time but it becomes smaller as prophase proceeds and eventually disappears just before the appearance of the spindle.

At metaphase the chromosomes arrange themselves normally on the equatorial plate, but become so closely crowded together that they are individually indistinguishable. The chromosomes divide longitudinally and, retaining their compact, crowded form, pass as two groups of daughter chromosomes to the poles. Interzonal fibers are evident at this time extending from one group of chromosomes to the other. After completing a short resting stage the cells divide again.

Meiosis

After completion of growth as normally occurs in meiosis the pri-

EXPLANATION OF PLATE I

FIG. 1. Spermatogonium in resting stage.

FIG. 2. Prophase of spermatogonium showing nine chromosomes. The longest is the unpaired chromosome.

FIG. 3. Primary spermatocyte in resting stage.

FIG. 4. Prophase of primary spermatocyte showing paired chromatin threads. Nucleolus in center.

FIG. 5. Prophase of primary spermatocyte with chiasma-like pairing of chromatin threads.

FIG. 6. Later prophase. One of the paired chromosomes in the center of the nucleus is broken and the broken ends attached to the ends of one of another pair of chromosomes.

FIG. 7. Diakinesis showing nine chromosomes. Eight of the chromosomes are paired and one is unpaired.

FIG. 8. Polar view of equatorial plate of first meiotic division.

FIG. 9. Lateral view of equatorial plate. Unpaired chromosome in center of spindle with ends directed toward poles.

FIG. 10. Anaphase. Unpaired chromosome lengthened and extending from one group of separating chromosomes to the other.

FIG. 11. Later anaphase showing constriction of unpaired chromosome at same level as cytoplasmic constriction.

FIG. 12. Telophase. Cytoplasm divided unequally by constriction. The unpaired chromosome extends from one nucleus to the other.

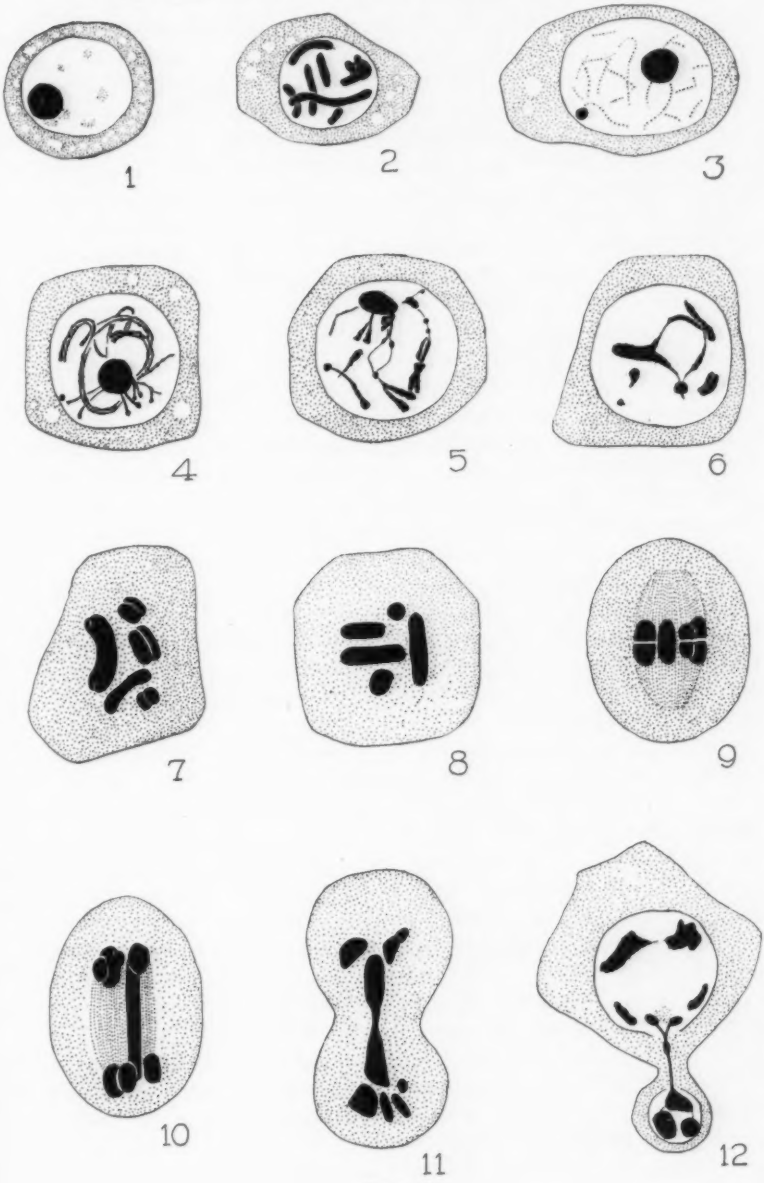


PLATE I

mary spermatocytes (Fig. 3) are about twice the size of the spermatogonia. One other difference between the two types of cells is the presence of a small nucleolus lying near the nuclear membrane of the primary spermatocyte in addition to the large nucleolus, which is like that found in the resting stage of the spermatogonia.

The prophase development is normal. The chromatin threads first appear as faint, beaded threads connecting rather large chromatin bodies (about half the size of the small nucleolus) that are scattered throughout the nucleolus. These granules disappear as condensation continues, leaving distinctly double, roughly beaded chromatin strands, which arrange themselves in loops extending outward from the nucleolus (Fig. 4).

The prophase stages showing the paired chromatin threads were studied with particular care in the hope of finding any unusual rearrangement of the chromatids. The segregation of genetic characters has never been determined definitely in any specific pattern in aphids due to the difficulty of breeding the gamic forms, though Shull (1925) found indication of a multiple factor inheritance of color. Therefore any prophase configurations that might indicate crossing over or other rearrangements of the chromatids, and thus genetic characters, would be of particular interest in this respect.

The intimate union of the chromatids at certain points, while keeping distinctly separate between these points, is particularly characteristic of chiasma formation. Recent cytological studies indicate that the formation of chiasmata is definitely correlated with crossing over so that the presence of one is evidence of the other. In Fig. 5 is shown a cell in which the paired chromatids have the appearance of characteristic chiasmata. A second example of chromatid rearrangement is shown in Fig. 6. One chromatid of a pair has become broken in the middle, the broken ends becoming attached to the ends of one chromatid of another pair. This might very easily result in a type of translocation. It is thus evident that there is in the prophase of the male maturation division the mechanism whereby portions of chromosomes can and do become rearranged, and which presumably results in a rearrangement of genetic characters.

The further contraction of these prophase strands ultimately forms smooth, rod-shaped chromosomes of unequal lengths (Fig. 7). They are thicker and shorter at this stage than in the corresponding stage of spermatogonial mitosis, and the size relationship between the individual chromosomes varies in one respect from those of the preceding division, namely, that in the spermatogonial mitosis the unpaired chromosome is the longest, while in the diakinesis of the first maturation division the

unpaired chromosome is exceeded slightly in length by one of the other pairs.

A difference of size relationship of the unpaired chromosome to the other chromosomes in the cell between the prophase of the spermatogonial mitosis and the first maturation division was described for one other aphid (*Eucraphis betulæ*) by Shinji (1931). He states: "Here [prophase of the first maturation division] the X-elements, four in number, are individually and collectively smaller than any one of the bivalents in the spermatocytes. When, however, we examine the equatorial plate of the spermatogonia . . . each X-element, except one, is nearly as large as any of the autosomes."

The arrangement of the chromosomes on the equatorial plate is normal in every respect. The unpaired chromosome is attracted to the plate to the same extent as the bivalents, and differs in this respect from the usual behavior of unpaired chromosomes in other forms, and in particular those of hybrids, in which the unpaired chromosomes are scattered over the spindle during metaphase and are not attracted to the plate until after the separation of the bivalents. A polar view shows only 5 chromosomes on the equatorial plate at this time, inasmuch as only 4 of the paired chromosomes and the unpaired chromosome are visible (Fig. 8). The unpaired chromosome is the second largest on the plate, though its identity can only be inferred from a knowledge of its size relation to the other chromosomes. This equatorial plate is a stage more often found than any other in the entire maturation and while the arrangement of the chromosomes differs occasionally, the unpaired X-chromosome is always on the plate. I believe, therefore, that it can be said with a high degree of assurance that the unpaired chromosome is subject to the same forces and to the same degree of regulation as the bivalents, and does not behave in an irregular manner. In a side view of the same stage there is no difficulty in determining the unpaired chromosome (Fig. 9). In the particular cell here drawn, the unpaired chromosome has further contracted and turned so that it lies perpendicular to the other chromosomes on the plate, and each end is directed toward one of the poles, while the paired chromosomes are just on the point of separating. The spindle fibers are visible but there are no centrosomes, which seems to be characteristic of all the cell divisions in this particular species.

The force exerted upon the paired chromosomes during anaphase, to draw them to the poles, must attract the two ends of the unpaired chromosome to the same degree as the bivalents, for each end of this chromosome is drawn toward one of the poles along with the segregating bivalents. This necessitates a stretching and lengthening of the un-

paired chromosome (Fig. 10), which seems as though it were destined to divide transversely in half along with the equal division of the cell, for a constriction appears in the middle of the lengthened chromosome and at the same level a constriction appears in the cytoplasm (Fig. 11).

The cytoplasm does not divide into two equal parts, for immediately after the appearance of the constriction it begins to slip toward one end of the elongated cell, and at the final division only a small amount of the cytoplasm is pinched off. This unequal division produces secondary spermatocytes of two sizes of which one is a large cell containing most of the cytoplasm and the other is a small cell containing very little cytoplasm (Figs. 12, 13, 14, 15).

The constriction of the lagging chromosome appears first halfway between the two ends, but gradually, as the cytoplasmic constriction slips to one end of the cell, the entire middle region of the chromosome becomes compressed while the ends get slightly larger as though part of the chromatin material were being forced into the ends by the constriction. At this time the lagging chromosome is much like a dumb-bell in appearance. Each end of the unpaired chromosome lies within the newly formed nuclear membrane of the daughter cells while the thin constricted middle portion extends through the cytoplasmic bridge that connects the two cells before division is complete (Figs. 12 and 13). The formation of the nuclear membrane is not complete in either cell at this time, for a gap is left open in both nuclei through which the lagging, unpaired chromosome extends (Fig. 12).

The appearance of the lagging chromosome at this time might easily lead one to conclude that it divides transversely, half going into each daughter cell. This does not occur, for just before the small cell is pinched off by the cytoplasmic constriction, the lagging chromosome is withdrawn from the small cell and goes in its entirety into the large cell (Fig. 14). Examples of this lagging chromosome going into the large

EXPLANATION OF PLATE II

FIG. 13. Telophase.

FIG. 14. Later telophase. Greater part of unpaired chromosome in larger cell.

FIG. 15. Large secondary spermatocytes having five chromosomes and small secondary spermatocytes having four chromosomes. Chromosomes split longitudinally in preparation for second meiotic division.

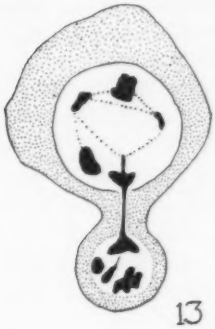
FIG. 16. Polar view of equatorial plate of second meiotic division.

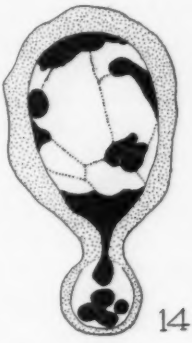
FIG. 17. Anaphase of second meiotic division.

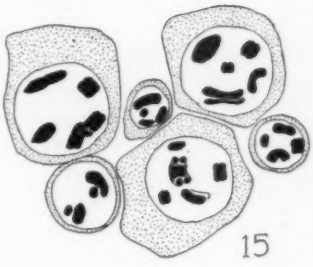
FIG. 18. Spermatids. Three degenerating, small secondary spermatocytes lying among spermatids.

FIG. 19. Later spermatid.

FIG. 20. Mature spermatozoa. Only a part of the tail has been drawn extending to the left.

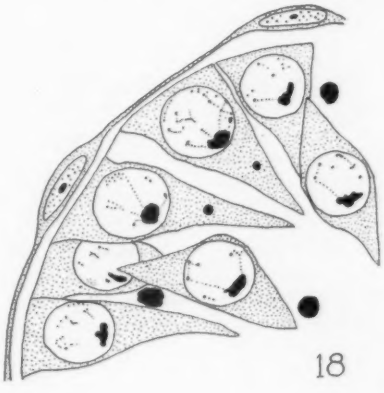

13


14


15

16

17


18

19

20

PLATE II

cells are very scarce, due no doubt to the rapidity with which the event takes place, though it is not difficult to find stages immediately before and after the final movement of this chromosome.

In the cell drawn in Fig. 14, showing the lagging chromosome going into the larger cell, the chromosomes are larger than those in the drawings of the preceding stage (Figs. 12 and 13). This might cause some question as to the accuracy of interpreting Fig. 14 as the stage following Figs. 12 and 13. The difference in size is probably not due to any fundamental difference between the two stages nor to a growth of the chromatin in the later stage, as might be supposed from these drawings, but rather to the swelling effect of the fixative used on the aphid from which Fig. 14 was taken. Gilson's fluid was used on this aphid and in general seemed to cause greater swelling than Navashin's fixative which was used on the aphids from which Figs. 12 and 13 were taken.

Immediately after the lagging chromosome has been drawn into the large cell, the small cell is severed completely from it by the cytoplasmic constriction. The two types of cells remain side by side throughout the cyst for some time. The large cells contain most of the cytoplasm and 5 chromosomes, while the small cells contain very little cytoplasm and only 4 chromosomes (Fig. 15).

After completion of the first division the large cells go through a short resting stage or interphase in which the chromatin becomes indistinct. Even in early telophase before the first division is complete the chromosomes begin to lose their well rounded, smooth appearance (Figs. 12, 13, and 14). The chromosomes spread out, seem to dissolve, and finally disappear altogether. The chromosomes in the large cells in Figs. 12 and 13 show this to some degree, but the best example is the chromosome lying along the periphery of the nucleus opposite the lagging chromosome in Fig. 14. The beaded or granular chromatin threads connecting the chromosomes, which were particularly evident during prophase of spermatogonial mitosis, are present again at this time. These connecting threads are characteristic of this aphid, for in some cases I have even observed them extending from one chromosome to the other on the equatorial plate of the first meiotic division, and they are particularly evident whenever the chromosomes are not condensed to their fullest extent, whether this be during prophase or during telophase. Stevens (1905) described similar connecting threads in *Aphis œnotheræ*.

The chromosomes in the large cells split longitudinally before the interphase, this being the first indication of the second maturation division which occurs immediately after the interphase. It seems likely that this split condition is retained through this resting stage, though during

it the individual chromosomes become lost to view, for at the first appearance of the chromatin threads in their recondensation their double nature is apparent. This return of the chromatin to a condensed condition follows normal prophase behavior, the chromatin becoming evident first as faint, thin threads which gradually thicken and shorten until they become typical chromosomes. There is a greater contraction at this time than during the prophase of the first meiotic division for the chromosomes that assemble on the equatorial plate of the second meiotic division are about one-half their former size (Fig. 16). However, all the chromosomes contract to the same degree so that each chromosome in any particular cell has the same size relative to the other chromosomes in that cell as in the preceding division. A direct comparison in one microscopic field of cysts containing equatorial plates of the second meiotic division with cysts containing the same stage in the first division shows clearly that the entire cell at the metaphase of the second division is smaller than the same stage in the first division.

During the anaphase of the second meiotic division (Fig. 17) the chromosomes divide along the longitudinal splits that were first visible in telophase of the preceding division before the interphase. This division is equational, none of the chromosomes lag, and each of the two equal cells that are formed contains 5 chromosomes, of which 4 are autosomes and one the unpaired or, as it has been termed by others, the X-chromosome. Interzonal fibers that stained no more densely than the spindle fibers are evident during the anaphase of this division. They extend from one group of separating chromosomes to the other.

After completion of the second maturation division the large cells develop into spermatids (Figs. 18 and 19) and eventually into mature spermatozoa (Fig. 20).

The small secondary spermatocytes remain scattered throughout the cysts among the large cells for some time after the first maturation division. The chromosomes split longitudinally and remain in the centers of the cell as if about to arrange themselves on the equatorial plate, but no spindle is formed and cell division never goes beyond this point. Instead the chromosomes become clumped together, while the cytoplasm begins to degenerate and gradually disappears, leaving a round mass of chromatin that stains very heavily. After the second maturation division the majority of the degenerating cells are found near the periphery of the cyst where they presumably have been forced by the movement of the large secondary spermatocytes during the division. The number of degenerating cells gradually decreases as development proceeds. A few are still evident lying among the developing spermatids (Fig. 18), but before the development of the mature spermatozoa the last trace of the small secondary spermatocytes is gone.

The presence of these two types of cells makes it possible to determine whether every division of a primary spermatocyte results in two unequal cells or whether two equal secondary spermatocytes are occasionally produced from one division. If in any one cyst after the first maturation division the number of large cells equals the number of small cells, the division of every primary spermatocyte must have been un-

TABLE I

Counts of male germ cells after the first and second maturation divisions showing the relative frequency of the two types of cells.

AFTER FIRST MATURATION DIVISION

Cyst	Large secondary spermatocytes	Small secondary spermatocytes
1	132	130
2	20	19
3	64	61
4	190	189
5	72	72
6	108	105
Total	586	576

AFTER SECOND MATURATION DIVISION

Cyst	Spermatids	Degenerating cells
1	372	212
2	235	94
3	263	108
4	302	166
5	408	201
6	467	236
Total	2047	1017

equal. And since only the large cells divide in the second division we should expect to find after that division that the spermatids were twice as numerous as the degenerating cells. If the division of the primary spermatocytes were not always unequal but occasionally resulted in two equal daughter cells, the above ratios would not be expected.

Counts were made of the secondary spermatocytes immediately after the division of the primary spermatocytes while each small cell was still lying close to its large partner. The results are shown in Table I. In the upper half of this table are listed counts of the large and small cells of 6 cysts directly after the first maturation division. In each cyst, as

in the total of the entire 6 cysts, the large cells and the small cells are about equally numerous. The proportion of the large cells in the total is 0.5043 ± 0.0146 (standard error, not probable), which is an insignificant deviation from 50 per cent. The lower half of the same table shows the counts made after the second maturation division. There is a greater variation in the individual cysts from the theoretical 2:1 ratio, but of the total number in the 6 cysts the proportion of large cells is 0.6680 ± 0.0085, which is only insignificantly different from two-thirds.

These proportions are so close to the expected ratios that there can be little doubt that the primary spermatocyte always divides unequally, resulting in large and small secondary spermatocytes in equal numbers, and that the large cells divide again, eventually producing viable spermatozoa, while the small cells degenerate. Schwartz (1932) reported similar results from a study of *Tetraneura ulmi* de Geer.

Maturation of the Parthenogenetic Female Egg

The reproductive system of the parthenogenetic female consists of a vagina, two short oviducts branching from the vagina, and a variable number of ovarioles (up to ten) branching from the oviducts. At the end of each ovariole is a germarium in which the germ cells are found.

Maturation begins in the germarium and is completed in the upper end of the ovariole to which the germarium is attached. The reproductive system of the most fully developed embryos enclosed within the adult parthenogenetic females proved to be the best place in which to study the maturation stages. The germaria within the embryos are made up of yolk-producing cells and germ cells, while each ovariole contains a maturing oöcyte and an embryo in the blastula stage of development. The oöcyte lies in the anterior end of the ovariole close to its attachment to the germarium and the embryo is found just posterior to the oöcyte. In Fig. 21 is shown a longitudinal section of a germarium and the end of the ovariole containing an oöcyte undergoing maturation. The embryo which usually lies posterior to this oöcyte is not shown. The germarium represented in the upper half of the drawing is separated from the oöcyte by a constriction of epithelial cells which form a covering for the germarium and make up the wall of the ovariole. These epithelial cells form a structure much like the finger of a glove of which the germarium occupies the extreme tip.

The germarium is an ellipsoidal (sometimes nearly spherical) structure in which are yolk-producing or so called " nurse " cells, and germ cells. The nurse cells are at the extreme tip of the ovariole and form a single-layered ball of cells surrounding a cavity filled with yolk, much like a typical bastula (Fig. 21).

Directly beneath the nurse cells in the drawing are shown 3 oögonia, two of which are in a prophase stage of oögonial mitosis, while the third, the center one, is beginning to increase in size and develop into an oöcyte. The oögonial divisions occur in this region of the germarium, but after each oögonium has completed its last mitotic division its descendant cells begin to grow and pass through the neck of epithelial cells into the ovariole. This epithelial membrane is elastic so that the opening is widened, allowing the enlarged germ cell to descend into the ovariole. The growth of the oöcyte is presumably due to the direct flow of yolk into the cell from the source of supply within the ball of nurse cells. A stream of yolk passes from this yolk supply to the oöcyte, and follows it down through the neck of epithelial cells into the ovariole, remaining connected until after the completion of maturation. In Fig. 21 this yolk stream can be seen extending from the yolk supply through the neck of epithelial cells to the tip of the ovariole. The drawing does not show a direct connection between the yolk stream and the oöcyte, but this can be plainly seen in Fig. 24. At the top of the latter drawing are represented a few of the epithelial cells which are a continuation of the ovariole wall and form a connection between the ovariole and the germarium. Between these two layers of cells is the yolk stream, flowing directly into the oöcyte by way of an opening in the cell membrane.

After entering the ovariole, the oöcyte continues to increase in size, as does the nucleus, and eventually becomes ellipsoidal in shape, due perhaps to the pressure exerted by the walls of the ovariole. It is impossible to count the individual chromosomes at this stage as they are wound about one another in a compact mass in the center of the nucleus (Fig. 21). After the oöcyte attains a size slightly larger than the germarium the nucleus migrates to the periphery, usually midway between the two ends of the ellipsoidal oöcyte. After reaching this point the nuclear membrane disappears and the individual chromosomes arrange

EXPLANATION OF PLATE III

FIG. 21. Upper half—germarium of parthenogenetic female showing cross-section of ball of nurse cells with yolk in center. Yolk stream extending from yolk supply to tip of ovariole. Three oögonia directly beneath nurse cells. Lower half—tip of ovariole with primary oöcyte.

FIG. 22. Primary oöcyte. Polar view of equatorial plate.

FIG. 23. Primary oöcyte. Lateral view of equatorial plate.

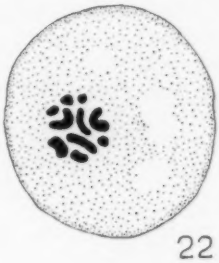
FIG. 24. Early telophase of maturation division and formation of polar body. At top of figure yolk stream entering oöcyte.

FIG. 25. Mature parthenogenetic egg with cleavage nucleus in resting stage. Polar body at right of figure between embryo and ovariole.

FIG. 26. Polar view and lateral view of cleavage mitosis in developing embryo.

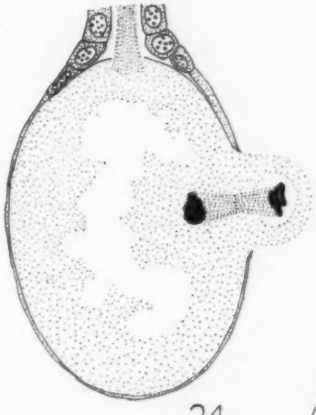
FIG. 27. Polar view of somatic mitosis in male.

21

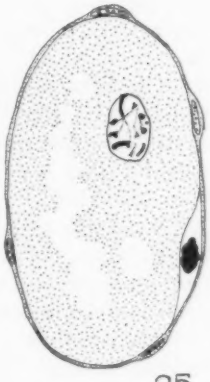


22

23

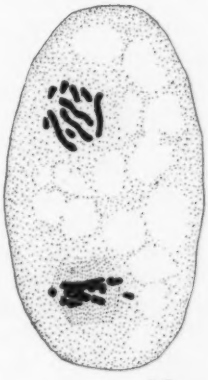


24



25

27



26

PLATE III

themselves on the equatorial plate of the spindle (Figs. 22–23). In Fig. 22 ten chromosomes, each slightly furrowed at the ends indicating a longitudinal split, are visible from a polar view of the equatorial plate. A side view of the spindle shows one pole lying close to the periphery of the oöcyte and the other pole directed toward the center of the egg. Ten chromosomes are also visible here, and in fact in all my observations of complete equatorial plates of this division, particularly the polar views, I never observed any variation of this number. The poles of the spindles lack centrosomes, as is true also of the cell divisions in the males. The chromosomes divide along the longitudinal splits previously noted, 10 chromosomes being extruded in the form of a polar body while 10 remain in the egg. A late anaphase of this division with characteristic interzonal fibers extending from one group of chromosomes to the other is shown in Fig. 24. It is impossible to count the individual chromosomes at this time because of their crowded condition. The polar body is pinched off and comes to lie between the ovariole wall and the egg membrane, as is shown in Fig. 25. The small amount of cytoplasm that surrounds the chromosomes of the polar body at the time of its separation from the egg has by this time degenerated and a small, darkly staining mass of clumped chromatin is all that is left. The polar body later sinks into the cytoplasm of the egg and is evident in the cytoplasm of the developing embryo until about the fourth or fifth cleavage division, after which time it is no longer visible, possibly having been absorbed.

As soon as the maturation division is complete and the polar body is extruded a nuclear membrane is formed around the chromosomes that remain in the egg. The chromosomes lose their identity as the resting stage of the first cleavage nucleus is formed, which remains near the periphery of the egg and soon undergoes the first cleavage mitosis. These first cleavage divisions are nuclear only, each of the daughter nuclei remaining close to the periphery of the egg so that a syncytium is formed of a peripheral row of nuclei around a central mass of clear cytoplasm. The cytoplasm eventually divides between the nuclei, forming a single layer of peripheral cells which usually surrounds two or three nuclei that are lying free in the central area of cytoplasm.

The individual chromosomes are very distinct in polar views of cleavage mitoses, which show ten chromosomes of five different sizes (Fig. 26). The five different sizes of chromosomes have the same size relationships as those described for the male germ cells, and also for the somatic cells of the male, as in Fig. 27 in which is shown an equatorial plate of a somatic mitosis taken from the wing bud of a male. The chromosomes of the female cleavage divisions differ from the male

germ and somatic chromosomes in that one member of the largest pair of chromosomes in the female is absent in the male. The absence of this chromosome in the male is the cause of the unpaired condition of one of the chromosomes of the male, which lags in maturation. Thus the condition in this species of aphid, *Macrosiphum solanifolii*, is similar to that found in phylloxerans by Morgan and for other aphid species by von Baehr and others in that the female has one more chromosome than the male. This chromosome and its partner, which form the longest pair in the female, are the so-called X-chromosomes. Two of them are found in the female while only one is found in the male.

Gamic females were not available for a study of their chromosomes, but it is safe to assume from analogy with other species of aphids that the chromosome number in the gamic female is 10 or the same as in the parthenogenetic female and that eggs are produced having the haploid number of 5 chromosomes. This assumption can be made with a high degree of certainty because in all the species previously studied the results have been the same. The disagreement has always been concerned with the fate of the lagging chromosome in the male. Because of the degeneration in the male of the secondary spermatocytes containing 4 chromosomes, all the viable spermatozoa contain 5 chromosomes and the individual that develops from the fertilized egg of the gamic female must necessarily contain 10 chromosomes, which is the female number.

Discussion

The controversy in aphid cytology has invariably been concerned with the fate of the unpaired chromosome or chromosomes, and the relative sizes and fate of the secondary spermatocytes. Morgan (1908), von Baehr (1908), Schwartz (1932), and others claimed that the unpaired chromosome acted as a sex-determining chromosome and went in its entirety into one secondary spermatocyte, while the other secondary spermatocyte receiving very little cytoplasm, was much smaller and degenerated. Jeffrey (1933) believed the previous work to be in error, stating that in *Aphis salicicola* and *Aphis populifoliæ* the division of the primary spermatocyte resulted in two cells of equal size, neither of which degenerated. The chromosomes were assorted equally between the two secondary spermatocytes and while he mentioned the presence of lagging chromosomes, which were similar to lagging chromosomes described previously by other workers, he did not refer directly to their behavior. He held that the lagging chromosome indicated the existence of hybridism in the aphid. Suomalainen (1933) likewise disagreed with the work previously done for he thought that the lagging

chromosome divided transversely, a small portion of one end being pinched off by the cytoplasmic constriction during the division of the primary spermatocyte. However, he agreed with Morgan and others that small secondary spermatocytes were formed which degenerated, and that the lagging chromosome acted as a sex-determiner. Suomalainen made no mention of Jeffrey's work.

My own work on *Macrosiphum solanifolii* is in direct opposition to that of Jeffrey. In this species there is an unequal assortment of chromosomes as the extra, unpaired, lagging chromosome goes wholly into one of the secondary spermatocytes, resulting in this secondary spermatocyte having one more chromosome than the other. The cytoplasmic division of the primary spermatocytes is also unequal so that secondary spermatocytes of two sizes are produced. The larger cells have the greater number of chromosomes and develop into mature spermatozoa, while the small secondary spermatocytes degenerate.

The only point in which Suomalainen's results differ from mine is that of the fate of the lagging chromosome. He states that it divides transversely, a small piece of one end being left in the small secondary spermatocyte. Suomalainen observed a small piece of darkly staining material in the small secondary spermatocyte which he assumed to be the pinched off end of the lagging chromosome. However, he was uncertain of this for he states: " . . . allerdings ist es schwer mit absoluter Sicherheit festzustellen, ob es wirklich ein Chromosom ist, da das Plasma auch andere mit Hämatoxylin sich dunkel färbende Körper enthält." In *Macrosiphum solanifolii* the small secondary spermatocytes contain no structure than can be interpreted as the pinched off end of the lagging chromosome.

Suomalainen states as further proof of the division of the lagging chromosomes that the chromosomes of the second maturation division are smaller than those of the first maturation division. The reduction in size is presumably not a result of division, however, for I find a similar shrinkage in *Macrosiphum solanifolii* and here the lagging chromosome goes entirely into the large cell and does not divide.

In order to overcome the genetical consequences of the loss of one end of a chromosome, Suomalainen assumes that the end of the lagging chromosome is an " Eliminationchromosom," by which he may mean that it is inert, while the part that goes into the large cell is the active part, that is, contains the genes. No active portion of the lagging chromosome can be regularly pinched off to degenerate in the small secondary spermatocyte, for if the lagging chromosome lost one end during meiosis there would be a deletion in the next generation. If this shortened chromosome again found its way into a male in the next gamic

phase of the cycle, as it normally would in 50 per cent of the males produced, it would suffer a further deletion during the maturation of the germ cells. With a continuation of this process, which would be inevitable in the normal chromosome cycle, the lagging X-chromosome would eventually disappear.

There are two means by which such a loss could be avoided, by fusion during fertilization of the large part of the X-chromosome furnished by the spermatozoön with a small part of the same type of chromosome which would be furnished by the egg, or by the regeneration of the lost end during the parthogenetic phase. The fusion with the large piece of the X-chromosome of a small piece of the same type of chromosome during fertilization is improbable, for a study of the entire chromosome cycle reveals no mechanism whereby such a small piece of chromatin could be produced. Regeneration of inert chromatin may be possible though that this could occur with chromatin containing active genes seems unlikely, and it is unknown whether the end of the lagging chromosome is active or inert. If regeneration of a part of a chromosome can occur, Suomalainen's interpretation of the dividing X-chromosome could be correct, otherwise it is questionable.

The conclusions resulting from this work are in complete accord with the early work of Morgan (1908–1915), von Baehr (1908–1909) and the more recent work of Schwartz (1932).

Summary

In *Macrosiphum solanifolii* the male has nine chromosomes, while the parthenogenetic female has ten chromosomes, the male lacking one chromosome of the longest pair present in the female.

During the spermatogonial mitosis the nine chromosomes divide equationally and none of the chromosomes lag.

Certain prophase configurations during spermatogenesis indicate a strong possibility of crossing over.

Four pairs of chromosomes and one unpaired chromosome are visible during diakinesis of the first maturation division in the male.

The unpaired chromosome is attracted to the equatorial plate along with the bivalents at metaphase, differing in this respect from univalents in hybrids.

The unpaired chromosome lags and becomes lengthened at anaphase while the bivalents are being drawn to the poles, and remains extended across the plane of division as much in one daughter cell as in the other until just before the cytoplasmic constriction separates the two cells completely, at which time the unpaired chromosome goes in its entirety

into one of the cells. Thus of the two cells produced by the first division, one contains five chromosomes and the other contains four.

The cytoplasmic division of the primary spermatocyte is also unequal, as the greater proportion of the cytoplasm goes into the secondary spermatocyte containing the five chromosomes. The secondary spermatocyte with only four chromosomes receives very little cytoplasm and is about half the size of the other.

The large secondary spermatocytes with five chromosomes divide again after a short interphase. The division is equational and none of the chromosomes lag, so that each of the two cells resulting from this division contains five chromosomes. These cells develop into spermatids and eventually into mature, viable spermatozoa.

The small secondary spermatocytes with four chromosomes do not divide again and eventually degenerate.

The large and small secondary spermatocytes are present in equal numbers after the first maturation division, which indicates that the division of every primary spermatocyte is unequal. After the second maturation division the large cells, which at this time are developing into spermatids, are twice as numerous as the small, degenerating cells.

The parthenogenetic female has ten chromosomes. Only one polar body is given off during maturation, and as the division is equational, ten chromosomes go out with the polar body and ten remain in the egg.

A comparison of the somatic chromosomes of the male with those in the parthenogenetic female shows that the male lacks one chromosome of the longest pair present in the parthenogenetic female.

Gamic females were not available for this study, but on analogy with other aphids they should contain ten chromosomes.

The conclusions resulting from this investigation support Morgan's work on phylloxerans in opposition to that of Jeffrey and Suomalainen.

BIBLIOGRAPHY

VON BAEHR, W. B., 1908. Über die Bildung der Sexualzellen bei Aphididae. *Zool. Anz.*, **33**: 507.

VON BAEHR, W. B., 1909. Die Oogonese bei einigen viviparen Aphididen und die Spermatogonese von Aphis saliceti, mit besonderer Berücksichtigung der Chromatinverhältnisse. *Archiv. f. Zellforsch.*, **3**: 269.

VON BAEHR, W. B., 1920. Recherches sur la maturation des oeufs parthénogenétiques dans l'Aphis palmae. *La Cellule*, **30**: 317.

JEFFREY, E. C., 1933. Meiosis in aphids. *Am. Nat.*, **67**: 79.

MORGAN, T. H., 1908. The production of two kinds of spermatozoa in phylloxerans—functional "female producing" and rudimentary spermatozoa. *Proc. Soc. Exper. Biol. and Med.*, **5**: 56.

MORGAN, T. H., 1909. A biological and cytological study of sex determination in phylloxerans and aphids. *Jour. Exper. Zoöl.*, **7**: 239.

MORGAN, T. H., 1910. The chromosomes in the parthenogenetic and sexual eggs of phylloxerans and aphids. *Proc. Soc. Exper. Biol. and Med.*, **7**: 161.

MORGAN, T. H., 1912. The elimination of the sex chromosomes from the male-producing eggs of phylloxerans. *Jour. Exper. Zoöl.*, **12**: 479.

MORGAN, T. H., 1915. The predetermination of sex in phylloxerans and aphids. *Jour. Exper. Zoöl.*, **19**: 285.

SCHWARTZ, H., 1932. Der Chromosomenzyklus von Tetraneura ulmi de Geer. *Zeitschr. f. Zellforsch. u. mikro. Anat.*, **15**: 645.

SHINJI, O., 1931. The evolutionary significance of the chromosomes of Aphididæ. *Jour. Morph. a. Physiol.*, **51**: 373.

SHULL, A. F., 1925. The life cycle of Macrosiphum solanifolii with special reference to the genetics of color. *Am. Nat.*, **59**: 289.

STEVENS, N. M., 1905. Studies in spermatogenesis with especial reference to the "accessory chromosome." *Carn. Inst. Wash. Publ.*, **36**: 1.

STILES, K., 1934. Normal butyl alcohol technic for animal tissues with special reference to insects. *Stain Technology*, **9**: 97.

SUOMALAINEN, E., 1933. Der Chromosomencyclus von Macrosiphum pisi Kalt. (Aphididæ.) *Zeitschr. f. Zellforsch. u. mikro. Anat.*, **19**: 583.

FURTHER EXPERIMENTS ON THE FEEDING OF CALANUS FINMARCHICUS[1]

JOHN L. FULLER AND GEORGE L. CLARKE

(Sarah Lawrence College, Bronxville, New York, and Harvard University, Cambridge, Mass.)

Introduction

The prominent position of copepods in the food cycle of the sea has caused various investigators to study conditions influencing their production—in particular their food supply. Since previous observations on the food relationships of copepods were meager and conflicting, a series of experiments was initiated at the laboratory of the Woods Hole Oceanographic Institution in an effort to throw light upon this problem (Clarke and Gellis, 1935).

In their previous work these investigators obtained most satisfactory results with *Calanus finmarchicus*. It was found that individuals provided with sea water which had been passed through filter paper lived and moulted in large numbers, whereas other individuals, provided with sea water from which all particulate matter had been removed by means of a membrane filter, failed to moult and eventually died. Since the copepods throve in water from which the larger constituents of the phytoplankton—chiefly diatoms—had been removed, it was evident that this species at least could live on the small pelagic organisms, or nannoplankton, and perhaps actually did so in nature.

The further experiments on *Calanus* about to be described were undertaken in order to test the whole question more thoroughly, and, it was hoped, to ascertain at the same time which of the many components of the nannoplankton are chiefly concerned. There exist in the sea many bacteria, unicellular algæ, and protozoans which are small enough to pass through the pores of the rather coarse filter paper used (Whatman No. 12). Since bacteria were expected to be numerically most abundant, we began our scrutiny of the nannoplankton by testing this group as a possible source of food for *Calanus*.

Extension of Experiment Using Continuously Flowing Media

In the first of the present experiments, it was proposed to control more carefully than previously the bacterial population present in the

[1] Contribution No. 92, Woods Hole Oceanographic Institution.

flasks by using measured amounts of known strains of bacteria and by keeping careful track of subsequent changes in the population. Using the same method as Clarke and Gellis, continuously flowing media were supplied to flasks in which copepods were confined. One flask received water filtered through a Berkefeld cylinder of porosity W (which was found to remove all bacteria and to be more convenient than the membrane filter used previously). A second flask received Berkefeld-filtered water which had been inoculated with a small amount of a mixed suspension of three types of common marine bacteria. A third flask was supplied with a large amount of the same bacteria in Berkefeld-filtered water. At the end of four days bacterial counts showed that the first flask did not remain sterile and the desired quantitative difference between the second and third flasks was not maintained. Since the bacterial population in the flasks was not satisfactorily controlled, a different procedure was obviously necessary, but the experiment was useful in demonstrating the extreme rapidity with which the bacterial situation can alter in culture flasks.

Attempts to Free Copepods of Bacteria

The difficulties cited above would have been avoided, had it been possible to remove all bacteria from the copepods before commencing the experiment. Attempts to wash the animals free of bacteria by passing through the flask in which they were confined about sixty liters of water in eight hours resulted in a reduction of the bacterial count from over 10,000 to under 10 per cc., provided the animals were transferred to a fresh sterile flask twice during the washing period. Encouraged by this great reduction, we constructed a special washing tube consisting of a test tube (12.5×2.8 cm.) to the bottom of which was sealed an elbow of small bore glass tubing (Fig. 1). A glass bead allowed water to enter from below, but prevented the escape of any copepods when the flow of water ceased. Sea water collected at about three hundred yards from shore was passed through filter paper and then through the Berkefeld filter. The continuous flow, which was maintained from the filter, was divided into two parts each of which ran through a cooling coil and then was introduced at the bottom of one of the special washing tubes suspended in a water bath at 12° C. The upper end of the washing tube was closed with a two-hole rubber stopper through which passed (1) an outflow tube, the mouth of which was covered with bolting cloth, and (2) a second tube containing a cotton plug, which was kept closed until the end of a run, when it was used to equalize the pressure in the washing tube with that of the atmosphere. The outflows of the

two washing tubes joined and were connected by way of two carboys to an aspirator which drew water through the whole system. A negative pressure of from 440 to 340 mm. of mercury as measured by a manometer in the system was maintained during the washing. Tests showed that this reduced pressure had no harmful effect upon the copepods.

Filter, cooling coils, washing tubes, and outflow tubes were sterilized

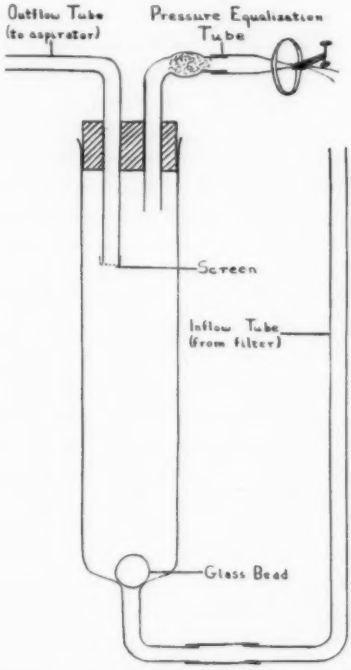


FIG. 1. Washing tube for removing bacteria from *Calanus*.

in an autoclave. Twenty *Calanus* were introduced into each tube and washed for two or three hours with twenty liters of sea water. At the end of the washing periods the animals were poured into flasks of sterile water cooled to 12° C. This water had been passed through a Berkefeld filter, and then heated in the flask for thirty minutes at 90° C. Bacterial counts made on these flasks shortly after adding the copepods amounted to from 0 to 12 bacteria per cc. After standing overnight

the counts rose to from 60 to 400 per cc. This concentration of bacteria we believe to be negligible in so far as the nutritional needs of copepods are concerned (see below).

The Availability of Bacteria in High Concentrations as Food for Calanus

Four lots of twenty animals each were washed daily by the method described above. Two of these groups, No. 1*a* and 1*b*, were each poured after washing into a 250-cc. flask containing 150 cc. of sterile water prepared as described above. Lot No. 2 was placed in paper-filtered sea water. Lot No. 3 was placed in water treated as for groups 1*a* and 1*b*, but subsequently inoculated with a loopful of bacteria [2] scraped from an agar slant. A bacterial count was made on each flask after the copepods had occupied it for approximately twenty hours. The animals were then transferred to the washing tube by means of a sterile wide-mouthed pipette. The copepods in all four groups were subjected to equal manipulation which did not appear *per se* to cause injury. A considerable number of animals were lost, however, by being caught on the sides of the tube during pouring. In reporting the results of the experiment these animals are not considered (Table I).

The numbers of bacteria in these flasks varied considerably, but were consistently very much smaller in flasks No. 1*a* and No. 1*b* than in flasks No. 2 and No. 3. During the first nine days both flasks No. 1*a* and No. 1*b* gave counts close to 500 per cc. Subsequently the counts rose markedly to average between 2,000 and 4,000 bacteria per cc., and on one day (forty hours after washing) were about 10,000 per cc. in each flask. In flask No. 2 the counts ranged from 50,000 to 800,000 per cc. and averaged 250,000. Bacterial counts in flask No. 3 fluctuated from 300,000 to 1,500,000 per cc. with an average of 600,000. From the nutritional point of view, flasks No. 1*a* and 1*b* may be considered sterile because, as will be shown below (Table III), the relatively small number of bacteria present would provide a negligible amount of nutriment. The concentration of bacteria in flask No. 3 was many times greater than has ever been found in the sea. In flask No. 2 small flagellates were present also, but time did not permit a quantitative estimation to be made.

The apparent paradox of the highest death rate occurring in the flasks best supplied with food may possibly be explained by the fact

[2] These bacteria were contributed by Dr. Selman A. Waksman. They belonged to a common marine species listed as No. 18 in his collection.

that in flask No. 2 six of the deaths (50 per cent) and in flask No. 3 two of the deaths (20 per cent) occurred during moulting, whereas in flasks No. 1*a* and 1*b* there were no indications of moulting. The copepods in flasks No. 2 and 3 were apparently receiving enough food to carry them to the moulting stage, but not enough to enable them to pass this critical period. It is possible that in flask No. 3 *Calanus* did not utilize the bacteria in suspension, but rather fed on the small amount of solid matter unavoidably added with the inoculum. The starved animals lived longer on the average than those with a little food, because they did not grow, and did not attain any critical stage. Since in the experiments of Clarke and Gellis *Calanus* moulted successfully when

TABLE I

Bacteria in high concentrations as food for **Calanus. Percentage of animals living after number of days indicated at left. Twenty animals at start.**

	Flask No.	1a	1b	2	3
	Treatment of sea water	Sterilized		Paper filtered	Bacteria added
	Accidental deaths	7	6	7	8
days					
0		100	100	100	100
3		80	100	100	88
6		64	100	87	75
9		57	93	69	67
12		54	93	38	50
15		54	64	8	33
18		31	43	8	17

supplied with paper-filtered water, the attendant conditions must have been more favorable in some respect. Possibly more food was made available by the use of continuously flowing water.

Two conclusions may be drawn from this experiment:

(1) *Calanus* will live without growing for over two weeks under starvation conditions.

(2) Bacteria, even in high concentration, are not an adequate food for *Calanus*.

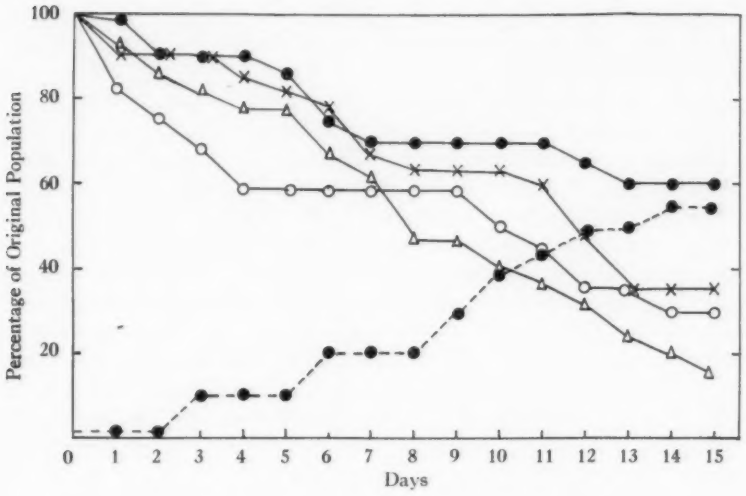
SEPARATION OF MICROÖRGANISMS INTO VARIOUS SIZES

Since bacteria were found to be unimportant as a source of food for *Calanus*, an attempt was next made to determine the size range of the organisms actually used as food by this copepod among the various other

types of nannoplankton. For this purpose the animals were placed in harbor water which had been passed through a graded series of filters as indicated in Table II.

Duplicate groups "*a*" and "*b*" were first set up in each series with twenty animals each. Some unknown factor, probably a contamination of the harbor water, caused a large number of deaths on the second and third days of the experiment. Consequently the copepods from each pair were united in one container, and a new group "*c*" started in each series. The toxic agent, however, probably influenced subsequent results, for the death rates in combined "*a*" and "*b*" (hereafter called "*ab*") are in every case higher than those in the corresponding "*c*" groups. Excluding the first three days, group "*ab*" ran for fifteen days and group "*c*" for eleven days. The animals, which were kept in covered glass dishes set in a water bath at 15° C., were transferred daily to clean containers of freshly prepared sea water. Samples of water were centrifuged from time to time, and microscopical examinations of the organisms thrown down were made.

In going from untreated water to water passed through finer and finer filters one finds (Table II and Fig. 2) a consistent decrease in the ability of *Calanus* not only to moult successfully but also to survive. This is especially true if adults are disregarded, since they survived equally well under all conditions of these experiments—probably because they have stopped growing and are no longer dependent upon a regular food supply. It might be concluded from these results that *Calanus* lives best on the larger planktonic forms—diatoms, peridinians etc.—which were found abundantly in the harbor water, and which were held back by the paper filters. Another interpretation is possible, however. Microscopic examination of centrifuged samples of the paper-filtered water revealed numbers of minute flagellated organisms, but there were even more of these in similarly centrifuged samples of unfiltered water. Evidently many of the smaller organisms were caught in the meshes of the filter of each grade along with the larger ones with the result that a purely qualitative separation according to size was not attained. Therefore, it is impossible to judge from these results whether the differences between the welfare of the copepods in the several experiments were due to quantitative differences in the supply of flagellates and other small organisms, or to qualitative differences in the types of organisms available. Since bacteria were abundant in all containers (Table II), it is again clear that they are not an adequate food for *Calanus*.



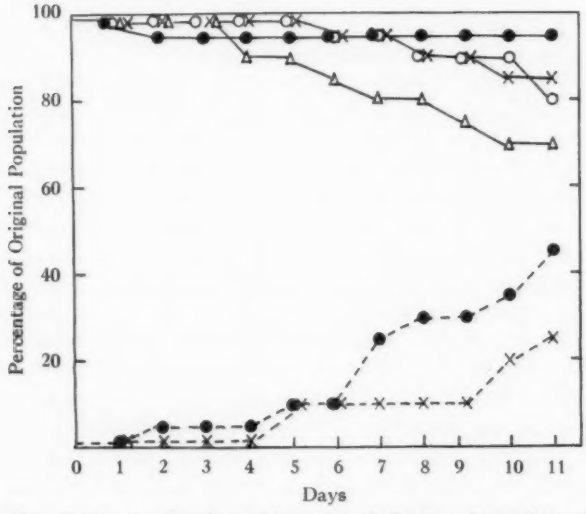


FIG. 2*a*. Series *ab*. Survival and moults of *Calanus finmarchicus* in water passed through different types of filters. Percentage of original population surviving shown by solid lines; percentage of original population moulting shown by broken lines.

Solid circles, unfiltered; crosses, Whatman 12; open circles, Whatman 40; triangles, Berkefeld W.

FIG. 2*b*. Same for series *c*.

TABLE II

Test as food for Calanus of microörganisms separated into various sizes.
Roman numerals refer to copepodid stages.

Series No.	Filter used	Population at start	Number of moults	Died during moult	Died, not during moult	Population at end	Bacteria per cc. on third day
1 *ab*	Untreated	5 IV	1	2	2	0	50,000
		12 V	10	0	2	1	
		3 VI	0	0	2	11*	
1 *c*		4 IV	4	0	0	0	
		14 V	5	0	1	12	
		1 VI	0	0	0	6	
2 *ab*	Whatman No. 12	2 IV	0	1	1	0	250,000
		15 V	0	6	5	4	
		5 VI	0	0	1	4	
2 *c*		5 IV	4	0	1	0	
		12 V	1	0	2	13	
		2 VI	0	0	0	3	
3 *ab*	Whatman No. 40	4 IV	0	1	0	0	560,000
		15 V	0	0	10	5	
3 *c*		4 IV	1**	1	1	1	
		12 V	0	1	1	11	
		4 VI	0	0	0	4	
4 *ab*	Berkefeld W	5 IV	0	1	4	0	400,000
		21 V	0	0	18	3	
		2 VI	0	0	0	2	
4 *c*		16 V	0	0	6	10	
		4 VI	0	0	0	4	

* Includes two males. All other Stage VI recorded are females.
** This moult occurred on first day, and was probably started when animal was captured.

THE NUTRITIONAL REQUIREMENTS AND THE FILTERING RATE OF CALANUS

Our search for the organisms which actually do serve as food for *Calanus* would be considerably narrowed if we could show that certain types are so sparsely represented in the sea that a copepod could not take in a sufficient number of them during the day to meet its nutritional requirements. The daily food requirements of a stage V *Calanus* during the summer have been calculated by Marshall, Nicholls, and Orr

(1935) on the basis of the oxygen consumption of a large number of individuals, as 0.013 mg. of carbohydrate or 0.005 mg. of fat. To obtain an approximate idea of the volume of water which a stage V *Calanus* would be required to filter to obtain this amount of nutriment, we have estimated the food content of the different elements of the phytoplankton and nannoplankton per unit volume of sea water at a station occupied on July 5, 1935, and located 7 miles south of the entrance to Vineyard Sound—a locality where *Calanus* occurred abundantly throughout the summer.

TABLE III

Daily filtering requirement. Volume of water containing sufficient organisms of certain types to supply the daily nutritive needs of a stage V *Calanus finmarchicus*.

Organisms	Number of cells	Calculated weight	Nutritive equivalent carbohydrate	Daily filtering requirement
	per cc.	*mg./cc.*	*mg./cc.*	*cc.*
Diatoms and peridinians V.S.	100	1.25×10^{-3}	1.4×10^{-4}	93
"Flagellates" V.S.	800	2.3×10^{-4}	4×10^{-5}	325
Bacteria V.S.	20,000	7.8×10^{-6}	1.3×10^{-6}	10,000
Bacteria	1,000	3.9×10^{-7}	6.5×10^{-8}	210,000
Bacteria	1,500,000	5.9×10^{-4}	9.8×10^{-5}	140

Notes: Composition of organisms assumed as follows:

	Percentage			
	Water and inert	Protein	Fat	Carbohydrate
Diatoms and peridinians	90	4	1	5
"Flagellates"	84	12	0.5	3.5
Bacteria	84	12	0.5	3.5

V.S.—Data based on quantitative analysis of water samples taken at the Vineyard Sound whistle buoy July 5, 1935.

If all three types of organisms served as food, the daily filtering requirement will be 72 cc.

The mass per cc. of diatoms, peridinians, and "flagellates" has been estimated from cell measurements on preserved material made by Miss Lois Lillick. The density of the living cells has been taken as equal to that of sea water. The mass of bacteria per cc. has been calculated from plate counts made by Dr. Margaret Hotchkiss. Each organism has been considered as a cylinder 0.5μ in diameter and 2μ long with the density of sea water. The analyses of Brandt (1898) were used as a basis for the assumed composition of diatoms and peridinians; those

of Buchanan and Fulmer (1928) were used for the bacteria. We have found no published analyses of flagellate composition, and have considered it as similar to that of bacteria. Since these analyses were based upon mixed plankton samples, the values assumed and the results obtained (Table III) must be regarded as rough approximations only. The volume of water has been calculated which would have to be filtered if the copepod lived exclusively on one type of organism in the concentration in which it existed at the station considered.

Up to the present time no measurements have been made to our knowledge of the volume of water *Calanus* can effectively filter per day.[3] As measured from the drawings of Cannon (1928), the filtering surface of the appendages has an area of 0.12 sq. mm. To filter in 24 hours the minimum of 72 cc. of water (Table III) an average rate of flow through the filter of 6.9 mm. per second would be required. Whether this is a reasonable value in view of the fact that the appendages were observed by Cannon to make 10 strokes per second is a matter for speculation.

A direct measurement of the filtering rate was attempted in some entirely preliminary experiments in which *Calanus* was allowed to "feed" for a certain number of hours in a suspension of carmine particles, the concentration of which was determined before and after the feeding period. The suspension was made up in sea water and filtered through absorbent cotton. The concentration of the fine particles remaining in suspension was measured with a hemacytometer. From 10 to 15 cc. of this suspension were placed in a small glass dish and cooled to 12° C. in a water bath. Five specimens of *Calanus* were introduced on a small lifter of bolting cloth. The amount of water added with the animals caused no appreciable dilution of the carmine suspension. At intervals the concentration of carmine particles remaining in suspension was determined, and an examination made for fecal pellets containing carmine. Such pellets remain intact and do not confuse the results. A control maintained to check the effects of settling showed that carmine is unsatisfactory for use over long periods, since it settles slowly, and gradually goes into solution. For short periods, however, these effects can be neglected without seriously affecting the results. We are searching for a more satisfactory suspension with which to continue work.

The equation used to calculate the amount of water filtered over a given period of time was derived as follows. Let: C = concentration of particles in the suspension; V = volume of water per copepod; N

[3] Harvey, Cooper, Lebour, and Russell (1935) report that "The copepod *Eurytemora hirundoides* has been observed by Lucas (private communication) to eat as many as 5,000 cells of the very small diatom *Nitzschia closterium* hourly, or 120,000 per day."

= number of particles per copepod; W = volume of water filtered per copepod. Then:

$$dN = -dWC,$$

$$dC = \frac{dN}{V},$$

$$-dWC = V\ dC,$$

$$W = V\ ln\ \frac{C_1}{C_2}.$$

C_1 and C_2 indicate respectively the concentrations of particles at the beginning and end of a period of observation. The amount of water filtered in twenty-four hours has been calculated for one series of experiments (Table IV). The discrepancy between the value calculated after approximately six hours, and that calculated on the basis of longer periods is probably due to the fact that errors in counting have a greater effect on the results when the reduction of particle concentration is small.

TABLE IV

***Filtering rate of* Calanus.** In each experiment 5 stage V *Calanus* were placed in 20 cc. of carmine suspension.

Experiment No.	Relative concentration carmine		Duration	Water filtered	Water filtered per day
	Start	Finish			
			hours	*cc.*	*cc.*
3	97	63	5.75	1.72	7.16
4	97	60	5.50	1.93	8.4
5	97	45	17.5	3.23	4.22
6	97	45	18.0	3.23	4.31
7	97	28	24.25	4.96	4.91
8	97	30	24.0	4.68	4.68
				Average	5.61

DISCUSSION

The results from these experiments on the filtering rate would appear to indicate that a *Calanus* cannot filter during the course of a day a sufficient volume of water to satisfy its nutritional needs if food organisms are actually as sparse as is apparently the case at the station considered. But little reliance should be placed as yet upon these entirely preliminary tests, although it is hoped that this method of investigating

the filtering ability of copepods will prove valuable when perfected. The figures do show, however, that the bacteria would have to be many times more concentrated than they have ever been found in the sea in order for enough of them to exist in a volume of water which a copepod could reasonably be expected to filter in a day. This conclusion together with the results of the feeding experiments described above seems definitely to rule the bacteria out as a source of food for *Calanus*.

If copepods feed on diatoms the filtering of a smaller amount of water would be necessary, according to our results, than would be the case if "flagellates" were used provided that the number of flagellates in sea water has been correctly estimated. The total volume of the small, delicate, and easily destroyed species in the nannoplankton may turn out to be greater than supposed when adequate methods for their quantitative enumeration have been developed.

There is no doubt that the ratio of diatoms to copepods in the water is sufficiently great to meet the nutritional needs of *Calanus* if they are suitable for food and sufficiently concentrated. At the station considered only one *Calanus* was caught for each 28 liters of water theoretically strained by the net. Even though other organisms compete for the same food the potential supply would seem adequate since the rapid reproduction of the phytoplankton during periods of activity is well known.

The fact that food organisms are not evenly distributed either spatially or chronologically must also be considered. *Calanus* appears to be adapted for prolonged periods without food, since individuals in our experiments lived for nearly three weeks under starvation conditions. We have calculated the theoretical time of survival of *Calanus* assuming that metabolism continues at a normal rate under conditions of starvation, and that the total fat content is available as a reserve source of food. The average weight of fat per stage V *Calanus* in August in Loch Fyne is 0.094 mg. (Orr, 1934). Since the daily nutritional requirement of each animal is 0.005 mg. of fat (Marshall, Nicholls, and Orr, 1935), the fat reserve would last nineteen days. In early May the fat content per individual averages 0.026 mg., while the nutritional requirement is 0.003 mg. This represents a nine-day reserve. These calculations compare favorably with the results of our experiments when individual differences between animals are considered.

Before summarizing we wish to point out the gaps in our knowledge of the nutrition of *Calanus*, gaps which must be filled before a complete quantitative discussion can be given. We do not yet know the exact types of organisms utilized by *Calanus*, nor the factors influencing the proportion of each type in its diet. Data concerning the chemical

composition of separate elements of the plankton are very meager, since analyses have been made on mixed catches, part of which may be unavailable for a particular species of animal. The effect of starvation upon the metabolic rate of *Calanus*, and the effect of environmental condition upon the filtering rate of this copepod are also unknown.

Summary

Calanus which had been rendered practically sterile by a system of washing failed to moult when placed in sterile Berkefeld-filtered sea water but remained alive for about two weeks. The introduction of bacteria even in high concentration did not enable the copepods to moult. Rough calculations indicate that the concentration of bacteria in the sea is much too low to satisfy the nutritional needs of *Calanus*.

Calanus was found to live and moult in sea water from which the larger planktonic organisms had been removed by a paper filter, although it throve better in unfiltered water. Whether this difference was caused by qualitative or quantitative differences in the food supply has not yet been determined.

Preliminary measurements on the rate of filtration of *Calanus* gave a result less than that calculated to be necessary for the copepod to obtain sufficient food if diatoms and peridinians are the sole source of supply. Since bacteria cannot be regarded as an important source of food, the other small and little known elements of the nannoplankton should be scrutinized, as their abundance in the sea may be greater than previously supposed.

Acknowledgment

The authors are indebted to Mr. Donald Zinn, who collected the animals used in these experiments, and to Mr. Sidney Cobb, who rendered technical assistance.

BIBLIOGRAPHY

Brandt, K., 1898. Beitrage zur Kenntniss der chemischen Zusammensetzung des Planktons. *Wiss. Meeresunt Kiel Komm.*, **3:** 45.

Buchanan, R. E., and E. I. Fulmer, 1928. The Physiology and Biochemistry of Bacteria. Chapter III. Williams & Wilkins, Baltimore.

Cannon, H. G., 1928. The feeding mechanism of the copepods, Calanus finmarchicus and Diaptomus gracilis. *Jour. Exper. Biol.*, **6:** 131.

Clarke, G. L., and S. S. Gellis, 1935. The nutrition of copepods in relation to the food cycle of the sea. *Biol. Bull.*, **68:** 231.

Marshall, S. M., A. G. Nicholls, and A. P. Orr, 1935. On the biology of Calanus finmarchicus. Part VI. Oxygen consumption in relation to environmental conditions. *Jour. Mar. Biol. Assn.*, **20:** 1.

Orr, A. P., 1934. On the biology of Calanus finmarchicus. IV. Seasonal changes in the weight and chemical composition in Loch Fyne. *Jour. Mar. Biol. Assn.*, **19:** 613.

THE BUFFERING POWERS OF NATURAL AND DIALYSED HELIX POMATIA SERUM

KATHLEEN GODWIN TERROUX

(From the Physiology Laboratory, Cambridge, and the Department of Zoölogy, McGill University, Montreal)

Redfield et al. have shown (1929) that the buffering power of reduced hemocyanin of *Limulus* is increased in the presence of salts below pH 8.0, at the expense of the buffering power between pH 8.0 and pH 9.0. The resultant increase in the effectiveness of hemocyanin as a buffer in the physiological range amounts to about 40 p.c. The present experiments indicate a similar effect of salts on the oxyhemocyanin of *Helix pomatia*. Attempts were made to obtain crystals of hemocyanin by dialysis, following Dhéré's method (1918) but in many attempts crystals were obtained twice only, and then in insufficient amount for the experiments. The hemocyanin was therefore purified by shaking the serum with chloroform, which precipitates a small amount of presumably protein material, but leaves the hemocyanin in solution, followed by dialysis of the filtrate to remove diffusible impurities. A comparison of the buffering powers of serum so treated with those of natural serum gives results qualitatively comparable with those of Redfield et al.

Methods

Serum

Active *Helix pomatia* were used. Each batch of snails was bled after two days starvation by pericardial and heart incision. To avoid contamination with slime from the foot glands, a large aperture was made in the shell above the heart region, with a bridge of shell left between this and the shell margin. By holding a cloth over the natural shell opening, serum uncontaminated with slime was obtained. For titration curves on fresh serum, the blood was filtered and kept at 0°–2° C. until used, which was always within forty-eight hours of its drawing. The blood for the experiments on purified hemocyanin was filtered, shaken up with chloroform, again filtered, and dialysed against distilled water at 0°–2° C. until the chloride test was negative. Before carrying out the experiments, the sample was repeatedly evacuated and brought into equilibrium with air.

Chloride Determinations

The chloride determinations were carried out by the method of Van Slyke (1923) on duplicate 3 cc. samples of blood. The concentration of chloride varied from 71.5 to 85.0 milliequivalents per liter, although the samples were made up of, and therefore averaged, the sera of 20 to 200 snails. Duval (1930) found the chloride content, calculated as NaCl, of *Helix pomatia* blood when the animals were active to be 3.60–4.75 gm. per liter, i.e., 61–81 milliequivalents, values close to those found in the present experiments. During hibernation, and during dry summer weather, when the animals have retreated into their shells, higher Cl concentrations occur.

Copper Determinations

The Cu content of the samples of dialysed and untreated serum was determined by N. W. Pirie, of the Biochemical Laboratory, Cambridge. The method (Pirie, 1931) depends on the catalysis by Cu of the oxidation of cysteine in the presence of H_2O_2. The accuracy is to about 3 p.c. with the concentrations of Cu found, 0.086–0.10 mgm. per cc. in the fresh serum. Dhéré and Burdel (Burdel, 1922) found Cu concentrations of 0.065–0.075 mgm. per cc. in the blood of hibernating *Helix pomatia*.

Titration Curves

Titration of the samples was carried out as previously described (Terroux, 1931) in the cup of the glass electrode vessel. Carefully standardised HCl or NaOH was added from standard 1 cc. burettes, fitted with finely drawn out glass tips to facilitate the accurate addition of small amounts of reagent. Two 0.5 cc. portions of each sample were used for a titration curve, to one of which base was added, to the other acid.

pH Determinations

The pH of the initial sample, and the pH after each successive addition of HCl or NaOH, were determined at 21° C., after the mixture had reached equilibrium. The glass electrode apparatus described by Kerridge (1926) was used, with 3.5 N KCl in the calomel electrodes and for the liquid junction with the experimental solution. Moisture leaks over the surface of the glass electrode were obviated by working at about two degrees above room temperature, a procedure which does away with the necessity of using paraffin oil for insulation. With titration curves carried out in the cup of the electrode in this way, the same KCl contact must be used during a whole series of acid or base additions. That possible diffusion of KCl does not affect the accuracy

of the determinations has been checked by the author in previous experiments (Pirie and Godwin Pinhey, 1929). Each glass electrode vessel was calibrated against a series of buffers covering the pH range under investigation, and the buffers checked by hydrogen electrode determinations. Before and after each run, the glass electrode vessel

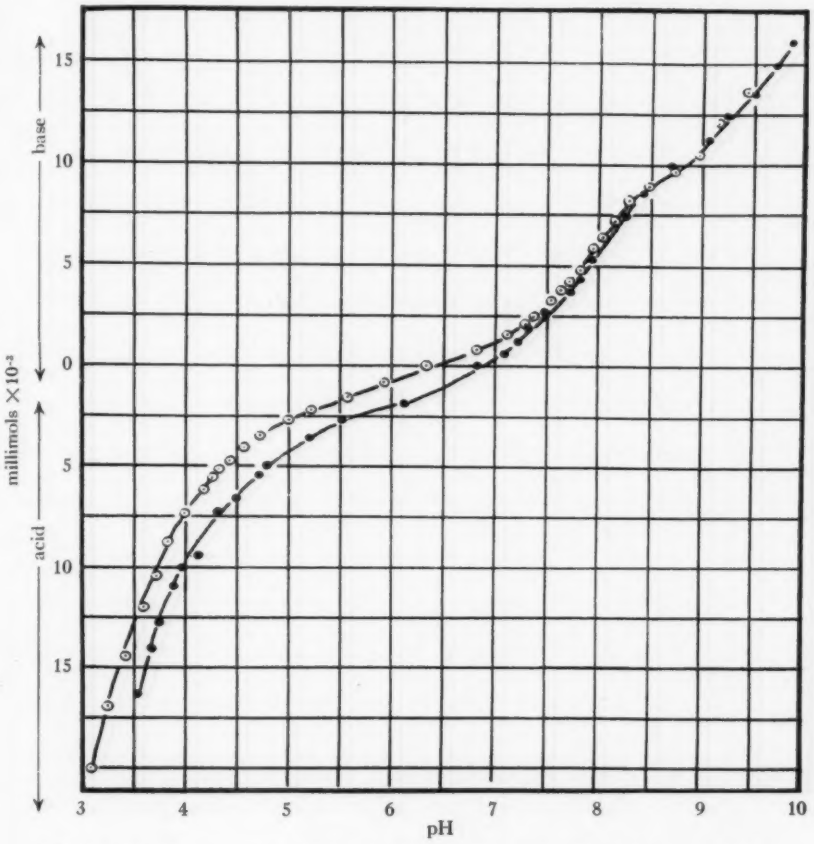


FIG. 1. Titration curves of dialysed serum; solid circle, May 20; open circle, July 23.

was checked against M/20 acid potassium phthalate, which has a pH of 3.97, and against phosphate buffer at pH 8.2. With titration curves carried out as described, each point on the curve represents a single pH determination.

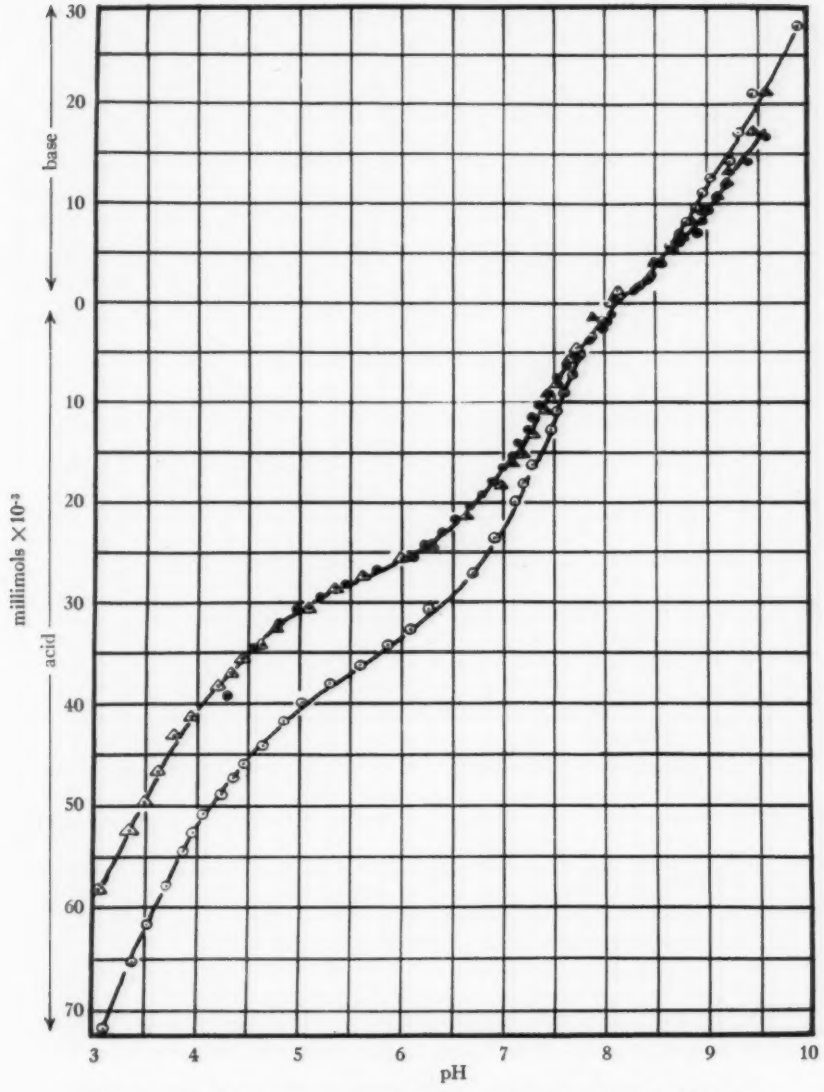


FIG. 2. Titration curves of natural serum. Open circle, July 8, 0.086 mgm. Cu per cc. sample. Triangle, July 21, 0.09 mgm. Cu per cc. sample. Solid circle, July 29, 0.10 mgm. Cu per cc. sample.

The results of the experiments are given in the figures below. For comparison, all curves are drawn on a basis of 0.1 mgm. Cu content. For the dialysed serum (Fig. 1) the buffering powers of the two samples (calculated to the same Cu and therefore the same hemocyanin con-

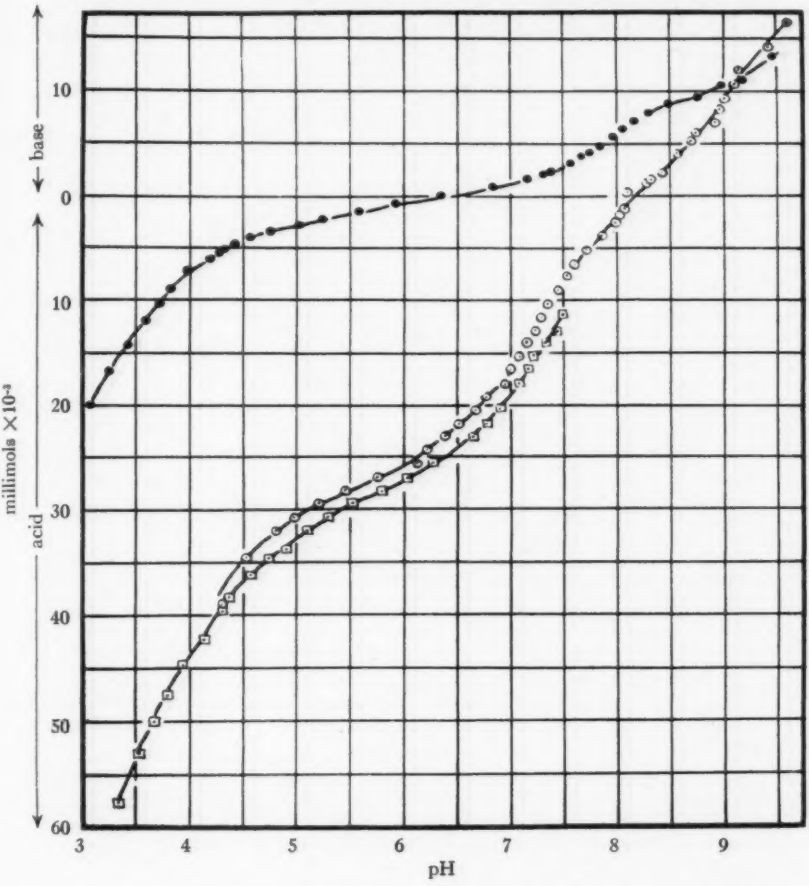


FIG. 3. Comparison of titration curves of dialysed and natural serum and test of repeatability of determinations. Solid circle, July 23, dialysed serum. Square, July 28, natural serum. Open circle, July 29, same serum as July 28.

tent) agree fairly closely, indicating that the only substance concerned in the buffering is the oxyhemocyanin. The natural serum curves fall into two groups when plotted on the 0.1 mgm. Cu basis. The curves

for the samples containing 0.1 and 0.09 mgm. Cu per cc. coincide. The chloride contents were respectively 85 and 80 milliequivalents Cl per liter. The curve for the sample containing 0.086 mgm. Cu and 71.5 milliequivalents Cl apparently has a higher buffering power, as shown by the increased steepness of the titration curve (Fig. 2). This may

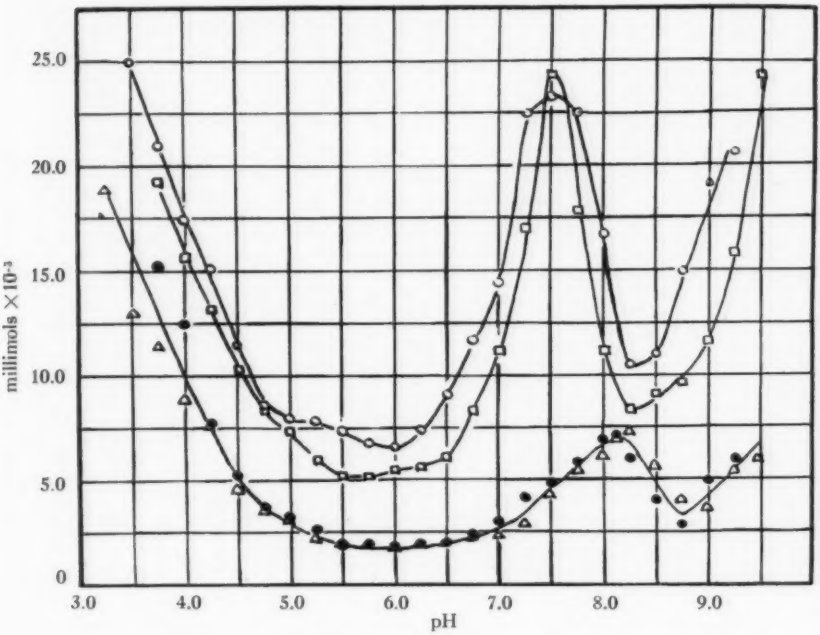


FIG. 4. Buffering powers of natural and dialysed serum. Open circle, natural serum, 0.086 mgm. Cu per cc. sample. Square, natural serum, 0.09 and 0.10 mgm. Cu per cc. sample. Solid circle, dialysed serum May 20. Triangle, dialysed serum July 23.

possibly be due to a higher carbonate content, as Duval (1929) has shown that dilution of the blood in the living animal results in a lowering of the Cl concentration, but an increased CO_2 content. In Fig. 3, the titration curve of dialysed serum is compared with that of undialysed. The two lower curves in this figure are from results on the same sample of serum on two successive days to test the repeatibility of the observations.

The buffering power of a solution is expressed by the equation

$$\beta = \frac{dB}{dpH}$$

(Van Slyke et al., 1922). For unit change in pH, β therefore is the amount of acid or base required to effect this change. The titration curves above give the buffering power of the hemocyanin containing solutions in terms of 0.1 mgm. Cu per cc. and 10^{-3} millimols acid or base. The slopes of these curves have been determined at intervals of 0.25 pH, and the results plotted in Fig. 4. The figure shows the higher buffering power of the serum containing 0.086 mgm. Cu per cc., but the correspondence of the shapes of the curves and the points of minimal and maximal buffering show that for all three samples of fresh serum the same buffering systems are involved.

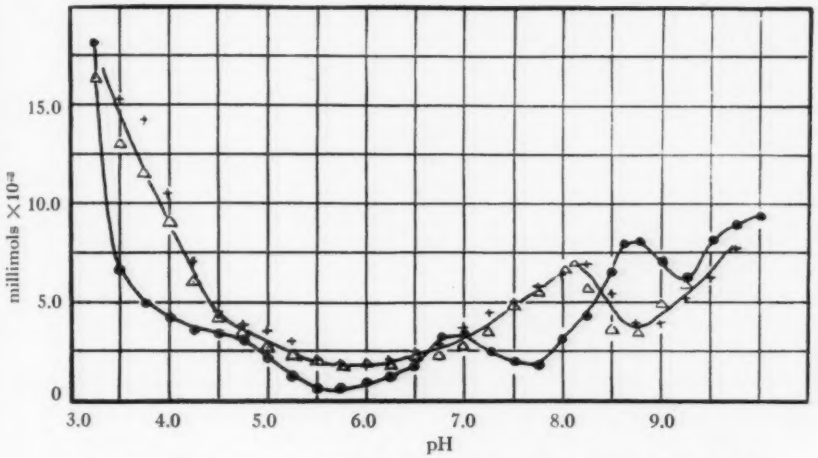


FIG. 5. Buffering power of Roche hemocyanin (represented by solid circle); of dialysed oxygenated serum May 2 (represented by cross); and of dialysed oxygenated serum July 23 (represented by triangle).

DISCUSSION

The chloroform-treated, dialysed solutions used in the experiments were not pure isoelectric oxyhemocyanin in solution, since the pH's of the two preparations were 6.82 and 6.34, and *Helix pomatia* hemocyanin has been shown to have an isoelectric point of 5.2–5.3 (Tiselius, quoted from Roche, 1931, and Stedman and Stedman, 1927). Proteins in the blood other than hemocyanin might be expected to exert a buffer effect, and would not be removed by dialysis. Cuénot (1892) found that in *Helix* blood there were no proteins precipitated by acetic, as in the case of crustacean and cephalopod blood, and further that all the protein

coagulable by heat was precipitated at one temperature, 69° C. He concluded that there was present only one albuminoid, the hemocyanin.

The concentration in the solutions of buffering substances other than hemocyanin can be estimated by a comparison of the buffering powers of the solutions used with that of a solution of pure isolectric hemocyanin. Roche (1931) has published a titration curve of such a solution of hemocyanin in which the nitrogen content was 78.9 mgm. His method was the same as that used in the present experiments, in that he added either HCl or NaOH to portions of the solution to obtain his curve. The pH was determined by the hydrogen electrode, so that presumably his findings are referable to reduced hemocyanin. After redrawing his published curve on a larger scale, the slope was determined at intervals of 0.25 pH. Roche has elsewhere (1933) published analyses of crystalline *Helix pomatia* hemocyanin, and gives a table averaging his results with those of Hernler and Philippi (1930). The N content is 15.14 p.c., the Cu content 0.250 p.c. Using these figures, Roche's buffering powers and those of my samples were calculated in terms of millimols $\times 10^{-3}$ acid or base and 0.1 mgm. Cu per cc., and plotted in Fig. 5. Although the two curves do not coincide, both show a minimum in buffering power between pH 5.5 and pH 6.0, a local maximum between pH 8.0 and 9.0, followed by a second minimum between pH 8.75 and 9.25. Moreover, the total buffer values are very close for the two curves. There can accordingly be present no considerable amount of buffer substance effective in the physiological range other than hemocyanin, so that the buffering power of the purified solutions dealt with can be considered close to that of pure oxyhemocyanin. From both Roche's curve and that resulting from the above experiments it appears that buffering due to hemocyanin is at a minimum at pH 5.5–5.75, on the alkaline side of the isoelectric point.

Roche deduces from his curve that hemocyanin behaves on the alkaline side of its isoelectric point as a polyvalent acid with two pK's, one between 7.1 and 8.8, and one at about pH 10.5. The maximum efficiency of a buffer is at that pH where equal amounts of the buffer are in the forms of the free acid and its alkaline salt, that is, when the pH of the solution equals the pK of the buffer. The pK can therefore be found by a determination of the point of maximum buffering. Roche's material gives a pK at 8.6. The corresponding pK of the present material (assuming that it is due to oxyhemocyanin only) appears to be lower, at about pH 8.2. Kerridge (1926) working on *Maia* blood, found that the maximum buffering of CO_2 by reduced blood was at pH 6.39, of the same blood oxygenated, at pH 6.205. Oxygenation had decreased the maximum by 0.185 pH units, a shift that

Kerridge accounts for by the fact that oxyhemocyanin is a stronger acid than reduced hemocyanin, and so competes more efficiently with CO_2 for the available base. A similar effect of oxygenation could account for the difference between the pH of maximum buffering, or pK of my solutions and that of Roche's reduced hemocyanin.

The interpretation of the rôle of hemocyanin in the buffering of the natural blood is more complicated. Unlike *Limulus,* where the blood contains only a trace of carbonate (Redfield et al., 1929) when the CO_2 pressure is reduced to zero, *Helix* blood from active animals has been shown to contain as much as 50 vols. p.c. CO_2 when the tension of this gas is zero (Nitzescu and Cosma, 1927). The high CO_2 content has been confirmed and extended by Duval (1930), who finds that in *Helix pomatia* when active there is an increase in the blood volume, correlated with a decrease in the chloride concentration and increase in the percentage of CO_2. Duval further finds that the total ionic concentration of the blood, as measured by the depression of the freezing point, can be accounted for by the sum of the depressions due to the chlorides and carbonates present. Phosphates occur only in a concentration of 1.9–3.0 mgm. inorganic phosphorus per liter (Duval and Portier, 1928). If the concentration of the chlorides and CO_2 compounds can account for the total freezing point depression, other substances of low molecular weight present must be in quantities sufficiently minute to be negligible in considering the total buffer action of the blood. The non-protein nitrogenous compounds present cannot have any appreciable buffer action. The blood of *Helix pomatia* contains only 0.11 gm. per liter non-protein N (Delaunay, 1912), about the same concentration as in *Limulus,* in which Redfield et al. (1929) found 0.12 gm. N non-coagulable by heat per liter blood. If, as Cuénot concluded, and a comparison with Roche's results indicates, the only protein present in the blood in significant quantity is hemocyanin, the buffering action of the blood must be accounted for by the carbonates and hemocyanin present.

Figures for the CO_2 content of *Helix pomatia* blood in equilibrium with air are given by Duval. For active snails, with chloride concentrations of 3.74–4.75 gm. NaCl per liter, the CO_2 content of the blood was 47.5–61.0 vols. per cent, an average of 54.4 vols., or 0.0243 millimols per cc. Since the chloride concentration of the natural blood in the present experiments was about the same, 71.5–85.0 milliequivalents per liter, as compared with 61–81 in Duval's experiments, a calculation of the buffering due to the CO_2 based on his figures should give an approximation of the extent of buffering to be expected from the CO_2 in the present samples. The Cu content of the samples was 0.10, 0.086,

0.09 mgm. per cc., so that the curves calculated for 0.10 mgm. Cu may be taken to represent the buffering of 1 cc. blood. The pH of the freshly drawn blood was 7.8–7.9. Peters and Van Slyke (1931) give a table of the percentage of CO_2 which will be present as carbonic acid, bicarbonate, and carbonate at pH 7.8, the pH of *Helix* blood. According to their figures 2 per cent will be carbonic acid, 1 per cent carbonate, and the rest bicarbonate. In changing to a pH of 7.0, the percentage increase in carbonic acid can be calculated from the equation

$$\frac{[H_2CO_3]}{[CO_2]} = \frac{1}{1 + \frac{K'}{[H^+]}}$$

to be from 2 to 11 per cent, or an increase of 9 per cent. This means that 0.0022 millimols bicarbonate will be converted into carbonic acid, a conversion requiring 2.2×10^{-3} millimols acid. But the sample of serum containing 0.1 mgm. Cu per cc. required 11.25×10^{-3} millimols to effect a similar change in pH. It is evident that the bicarbonates in themselves cannot account for more than about 20 per cent of the total buffer action of the blood. Further, if the CO_2 compounds were exerting the major buffer effect, the maximum found for the blood at pH 7.5 would be inexplicable, since the maximum buffering of a bicarbonate-carbonic acid buffer is at pH 6.1.

Comparison of the buffer curves in Fig. 4 shows the greater buffering power of the untreated blood. The buffering is not only greater over the whole range investigated for solutions containing the same amount of hemocyanin as in the dialysed preparations, but the pH at which maximum buffering occurs is at 7.5 for the natural blood, at pH 8.2 in the purified solutions. The only buffer substances found in any quantity in the blood are hemocyanin and bicarbonates. The sum of the independent buffering of these two cannot account for the total buffer action of the blood. This and the shift in the pH of maximum buffering must then be attributed to alterations in the buffering power of the hemocyanin brought about in the presence of diffusible substances—of which those present in predominant concentration are the chlorides.

Summary

Using the glass electrode for pH determinations titration curves of oxygenated fresh and dialysed *Helix pomatia* blood were determined.

From the slopes of these curves, buffering power was determined at intervals of 0.25 pH, and curves of the buffering powers drawn over the range pH 3.5–9.5

The fresh blood has a higher buffering power over the whole range, the difference becoming most marked at pH 7.5, where the curve for the blood shows a local maximum buffering. A similar maximum for the dialysed blood occurs at pH 8.2.

The bicarbonates present can account for about 20 per cent of the total buffering of the blood. Evidence is given that the remaining 80 per cent of the buffering is attributable to hemocyanin, and that the buffering power of the hemocyanin is augmented by the presence of salts.

REFERENCES

BURDEL, A., 1922. Thesis. Fribourg, Switzerland.

CUÉNOT, L., 1892. *Arch. de Biol.*, **12**: 683.

DELAUNAY, H., 1912. *Compt. rend. Soc. de Biol.*, **73**: 492.

DHÉRÉ, CH., 1918. *Compt. rend. Acad. Sci.*, **146**: 784.

DUVAL, M., 1929. *Compt. rend. Acad. Sci.*, **188**: 104.

DUVAL, M., 1930. *Physicochim. Biol.*, **6**: 346.

DUVAL, M., AND P. PORTIER, 1928. *Compt. rend. Soc. de Biol.*, **99**: 1831.

HERNLER, F., AND E. PHILIPPI, 1930. *Hoppe. Seyler's Zeitschr. f. physiol. Chem.*, **191**: 23.

KERRIDGE, P. M. T., 1926. *Jour. Sci. Inst.*, **3**: 404.

KERRIDGE, P. M. T., 1926. *Jour. Physiol.*, **62**: 65.

NITZESCU, L. I., AND L. COSMA, 1927. *Compt. rend. Soc. de Biol.*, **97**: 1110.

PETERS, J. P., AND D. D. VAN SLYKE, 1931. Quantitative Clinical Chemistry.

PIRIE, N. W., AND K. GODWIN PINHEY, 1929. *Jour. Biol. Chem.*, **84**: 321.

PIRIE, N. W., 1931. *Biochem. Jour.* **25**: 1565.

REDFIELD, A. C., G. HUMPHREYS, AND E. INGALLS, 1929. *Jour. Biol. Chem.*, **82**: 759.

ROCHE, J., 1931. *Compt. rend. Soc. de Biol.*, **107**: 1146.

ROCHE, J., 1933. *Compt. rend. Soc. de Biol.*, **114**: 1190.

STEDMAN, E., AND E. STEDMAN, 1927. *Biochem. Jour.*, **21**: 533.

TERROUX, K. GODWIN, 1931. *Jour. Physiol.*, **71**: 323.

VAN SLYKE, D. D., A. B. HASTINGS, M. HEIDELBERGER, AND J. M. NEILL, 1922. *Jour. Biol. Chem.*, **54**: 481.

VAN SLYKE, D. D., 1923. *Jour. Biol. Chem.*, **58**: 523.

SEX INVERSION IN TEREDO NAVALIS AND ITS RELATION TO SEX RATIOS

B. H. GRAVE AND JAY SMITH

(From the Marine Biological Laboratory, Woods Hole, and DePauw University)

In two important papers on the sexual cycle in *Teredo navalis*, Coe (1933 and 1934) has undertaken, among other things, to explain the observed predominance of females over males in adult life. This excess of females over males has been reported by various investigators to be as high as 97 per cent to 99.7 per cent (Nelson, 1922). Professor Coe, on the other hand, finds himself in practical agreement with Quatrefages (1849), who recorded observation of 8.6 per cent males in *Teredo norvegicus*. From various data, direct and indirect, Coe has concluded that there are 9 per cent males in adult populations of *Teredo navalis* after the middle of the breeding season. He believes, in other words, that approximately 10 per cent of all individuals remain permanently males. These he calls "true males." The others are considered to be protandric females so that the adult population would ultimately appear to be 90 per cent females.

A reinvestigation of sex ratios in the shipworm, *Teredo navalis*, an account of which is given in this paper, has therefore been made in response to the conclusion reached by Coe (1933) that females in this and other species of shipworm greatly outnumber the males. This conclusion or generalization forms the background and foundation of his theory of protandry, as applied to this species, which, stated briefly, assumes that all young teredos develop first a male phase during which the gonads produce functional sperm. This is followed promptly by a female phase which, according to the theory as first enunciated, persists during the remainder of the life of the individual. This theory, and especially its implications, are so definitely in conflict with observations of sex ratios in *Teredo* published by the senior author of this paper (Grave, 1928) that a reinvestigation was undertaken during the summer of 1935; particular attention being given to full-grown individuals which, in accordance with Coe's theory, should ultimately be 90 per cent females.

In a later note in *Science* (1934) Coe indicates that at the beginning of the second spawning season there may be a second male phase similar to the first, but that it also is brief in duration and affects only the oldest individuals of the preceding season which have already passed through

male and female phases. The reorganization of the gonads of this considerable group of young shipworms during the winter and early spring serves to increase greatly the ratio of males to females so that at the opening of the breeding season it is estimated to be four or more males to one female in the whole population. Most of these presumably transform into females again early in the second spawning season. He therefore concludes there is a great preponderance of females over males in adult life. His data show that this change from the male to the female phase takes place mainly during late May and early June so that the sex ratio is reversed at the opening of the second breeding season, the females predominating in a ratio of forty-eight males to one hundred females (Coe, Table I, 1933).

Our observations are not in opposition to Coe's conclusion that there is an early male phase but to the second stipulation that the female phase which follows persists during the entire adult life of the individual with the exception noted. This is, in fact, a necessary or fundamental postulate to account for the supposed great preponderance of females over males in adult life. As we understand his argument, this is one of the chief facts to be accounted for by any theory and constitutes the background of the theory of protandry as applied to this species, particularly in the first article (Coe, 1933). We find, on the contrary, that sex inversion continues to occur throughout the life of an individual and that in any shipworm population of adult animals there is no such significant difference in numbers of males and females. The problem at issue, therefore, is whether or not protandry is the correct term to apply to the sexual cycle of *Teredo* and whether as a consequence the adults are nearly all female or whether the sexes are more nearly equal in number.

A second purpose of our present study was an investigation of sex inversion in Mollusca in general, which has received considerable attention in recent years beginning with a paper on *Crepidula* by Gould (1917). A sufficiently extensive study of the peculiar hermaphroditism which occurs in various species of mollusk should throw light on the problem of the determination or differentiation of sex as influenced by environmental and heredity factors.

Sex Ratios in Teredo

A male may be defined, for the purposes of this study, as an individual which is producing active sperm in abundance and which, for the time being, is not liberating fertilizable eggs. In addition to such males there are a few (not rare) adult individuals which are hermaphro-

ditic in that they are carrying embryos in their brood pouches in various stages of development and which at the same time are producing active spermatozoa. In some of these, both eggs and spermatozoa intermingle in the lobules and ducts of the gonads. (See Figs. 3 and 4.) These individuals are, for the time being at least, truly hermaphroditic and provide every condition requisite to self-fertilization. A very few individuals are producing neither eggs nor spermatozoa, the gonads being empty and apparently collapsed or "spawned out." These are designated in the table as not spawning. They may be found at the height of the

TABLE I

Sex ratios in Teredo navalis

Date of collection	Character of individuals examined	Females	Males	Hermaphroditic	Not spawning
June 28	General population	49	11	0	5
" 29	Large specimens	23	6	1	0
" 29	General population	68	34	0	12
" 30	" "	10	7	0	0
" 28 to 30	(Totals for June)	150	58	1	17
July 1	General population	65	53	15	4
" 3	Large specimens	8	5	1	0
" 5	" "	28	27	4	0
" 6	" "	6	8	3	0
" 6	Medium large specimens	11	4	1	0
" 6	Small specimens	6	4	0	0
" 7	Large specimens	11	8	2	0
" 19	" "	9	3	0	1
" 20	" "	3	4	3	0
" 1 to 20	(Totals for July)	157	115	25	5
August 1	Large specimens	25	27	7	5
" 8	" "	19	25	4	2
" 27	" "	2	3	1	0
" 1 to 27	(Totals for August)	46	65	12	7
	Totals for entire season	353	238	27	29

breeding season in July but are apparently more numerous in August. Some of these are parasitised and injured by a ciliate or a sporozoan, both of which are of common occurrence in the gonads of *Teredo*. To complete the classification, there are many true females in which few or no spermatozoa or spermatogonia are visible and true males with no ovocytes in the gonads. These so-called "true males" are far more numerous in a population of adult *Teredo* than has been supposed and apparently more abundant at the end of the breeding season than earlier.

(Fig. 1.) Coe estimates them at approximately 10 per cent of the population.

Table I contains the results of examinations of living animals made during the present summer according to the above classification. It should be noted that the column labelled " males " includes, besides true males, many whose gonads contain ovocytes in various stages of development, and varying in number from a few scattered ovocytes to a complete layer of them in the epithelial wall of the lobules of the gonad. A male, according to this classification, as stated above, is an individual which is liberating abundant active spermatozoa and no eggs. In the same way some individuals classed as females in the table have a few or numerous spermatogonia among the ovocytes but are not for the time being shedding active spermatozoa. In these individuals either the male or the female phase is predominating to the exclusion of the other.

The teredos which were examined this summer were all taken from timbers placed in the water between August 1 and August 10, 1934. Those marked " General Population " were taken from crates made of $1 \times 3''$ boards and were crowded and relatively small. Those marked " Large Specimens " were taken from $2 \times 4''$ stakes and were large individuals, having grown under good conditions. They may be considered adults ten or more months old. The point of importance is that we are dealing with adult animals of the second season and not with young animals.

The tabulated data show that males may increase in number rather than decrease during the course of a breeding season and in no sense do they decrease to the vanishing point or to 10 per cent as required by the theory of protandry. No young teredos of the present season are included in this study but only those that entered the wood last year.

Tabulations of the ratios of the sexes made in former years (1923, 1924, and 1925) from which representative data were published (Grave, 1928), show also a preponderance of females over males but not to an extent such as has been frequently reported. A high ratio of males prevails whether large individuals are selected or whether the entire population is considered. Moreover, except in June when females are relatively abundant, the ratio of the sexes remains nearly the same throughout the breeding season but with a definite increase toward maleness. The conclusion is, therefore, warranted that both in the general population and in adult individuals the sex ratio of *Teredo* at Woods Hole is not less than 30 and may be as high as 50 per cent males.

Sex Inversion in Teredo

A bisexual condition of the gonads of various gasteropods and lamellibranchs has been observed by several investigators and it is generally believed that it is not a permanent condition but rather that the same individual may function as a male at one time and later become a female. This inversion of the sexes may take place several times during the lifetime of an individual as, for example, in the California oyster, *Ostrea lurida* (Coe, 1933). During the period of change of sex in *Teredo* the gonad appears hermaphroditic and sometimes contains active spermatozoa and fertilizable eggs as well as developing ovocytes and spermatogonia. This condition of true hermaphroditism has frequently been observed to obtain in *Teredo* for a short period just at the time of change from the male to the female phase and from the female to the male phase. Usually, however, one sex predominates and although both ovocytes and spermatogonia may be present, only one is maturing into functional gametes. Sections of a large number of teredos, therefore, show all gradations between maleness and femaleness and some that appear to be entirely male or entirely female. We, in fact, have found every type of gonad described by Coe and some even more convincing cases proving inversion of the sexes. These special cases are described below.

A careful study of adult individuals leads us to doubt if there is such a thing as a true male or a true female *Teredo* in the sense that it remains permanently one sex, as postulated by Coe, who agrees that sex inversion in true males may ultimately take place (Coe, 1933). It is evident from these studies that full-grown teredos show the same sex inversion that Coe has recognized in the younger animals. We believe the condition in *Teredo* is strictly comparable to that he described for the California oyster and to the snail, *Valveta tricarinata* (Furrow, 1935), rather than that of *Crepidula* in which an early male phase is apparently followed by a permanent female phase.

There is abundant evidence that a female *Teredo* may spawn two or three times and then rapidly change to a functional male. Several fully-grown individuals which were carrying both veliger larvæ and cleaving

Fig. 1. Section of the gonad of an individual in the male phase. Spermatozoa and spermatogonia abundant. Little, if any, trace of ovocytes visible.

Fig. 2. Section of the gonad of an individual in the female phase. The small nuclei which surround the eggs belong to nurse cells. There are few if any spermatogonia in this gonad at this time.

Fig. 3. Section of an hermaphroditic gonad. It is changing from the female phase to the male phase. The walls of the lobules are composed of proliferating spermatogonia and the cavities are filled with spermatozoa and free ovocytes. This gonad is in the active male phase, and after the last of the ovocytes have passed to the exterior it will appear to be wholly male.

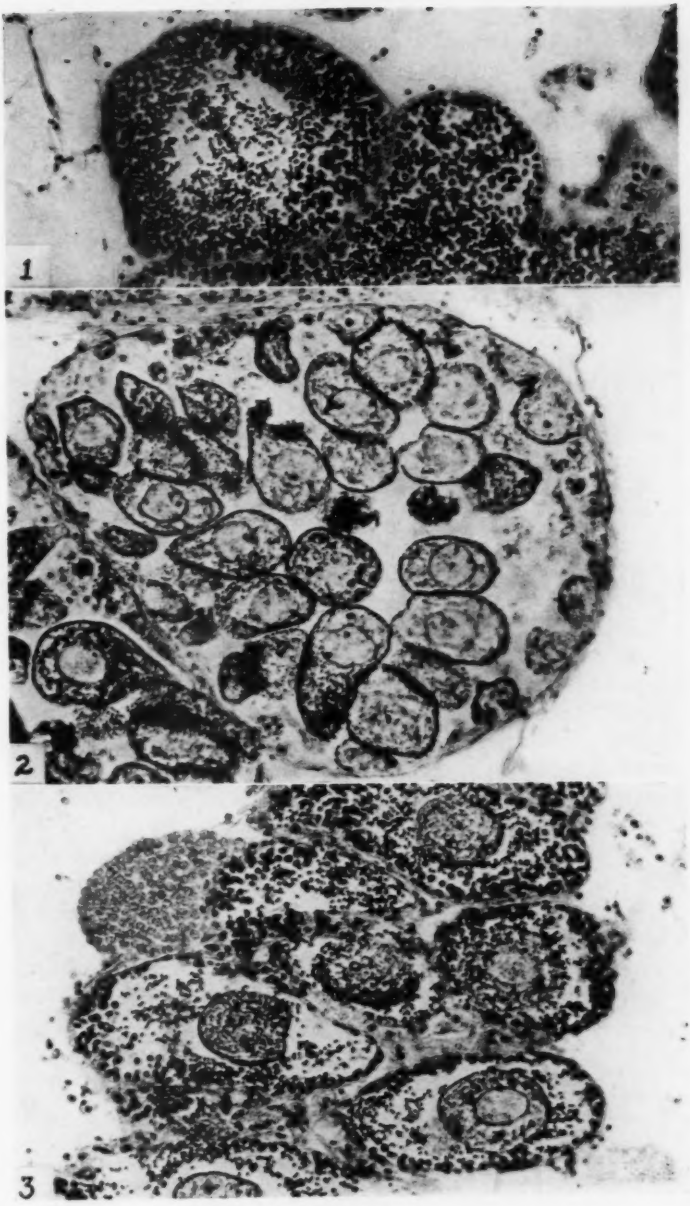


PLATE I

eggs in their brood pouches were at the same time producing active spermatozoa. The gonads of these individuals, when sectioned and stained, were seen to be functional testes with little trace of ovocytes remaining. These several examples of this type are sufficient to prove that there is sex inversion in adults from female to male. Other cases were frequently observed in which the lobules and ducts of the gonads contained free ovocytes with germinal vesicle intact while the walls of the lobule consisted of spermatogonia and spermatids. At the same time there were abundant spermatozoa free in the lumen surrounding the eggs. (Fig. 3.) That is, of course, a truly hermaphroditic condition, but since there are no large ovocytes in the wall of the lobules it is evident that the male phase is in the process of establishment from an antecedent female phase. Figure 4 shows the reverse condition in which ovulation is beginning while the lumina of the lobules of the gonad are still filled with spermatozoa. The last of the spermatogonia and spermatids are disappearing. These examples indicate that the functional change is accomplished rapidly because, in the first case, cleaving eggs in the brood pouches indicate their recent arrival there, yet the gonad had already become exclusively male. There is abundant evidence also that the hermaphroditic condition in some individuals is of short duration and that dominance of the male or female condition is the rule. Relatively few individuals are extruding both eggs and spermatozoa at the same time. There may be some truly hermaphroditic individuals but it appears more likely a temporary transitory condition of brief duration. The "spawned out" condition in which no gametes are visible in teased preparations of gonads, when not caused by parasites, is apparently a transition stage between sexual phases.

Figure 4 and possibly Fig. 5 represent types of gonads in which the male phase is coming to a close. The ovocytes in the latter are still small though abundant; Fig. 6 shows a condition in which the male phase is active in that the wall is composed largely of spermatogonia and spermatids while the lumen is filled with spermatozoa. A few scattered, half-grown ovocytes are seen in the wall which will presumably replace the spermatogonia. They probably remain quiescent during the active male phase. Figure 1 represents the male phase in which no ovocytes

FIG. 4. Section of an hermaphroditic gonad which is changing from the male phase to the female phase. The lumen of the lobule, shown in the photograph, is filled with spermatozoa and a few dividing spermatogonia are visible between the developing ovocytes. The male phase is almost completed.

FIGS. 5 and 6 are sections of gonads in the active male phase. The lumina of the lobules are filled with spermatozoa and the walls contain dividing spermatogonia and spermatocytes. Small scattered ovocytes are visible in the wall of the gonad and these, presumably, will ultimately replace the spermatogonia and transform the gonad into an ovary.

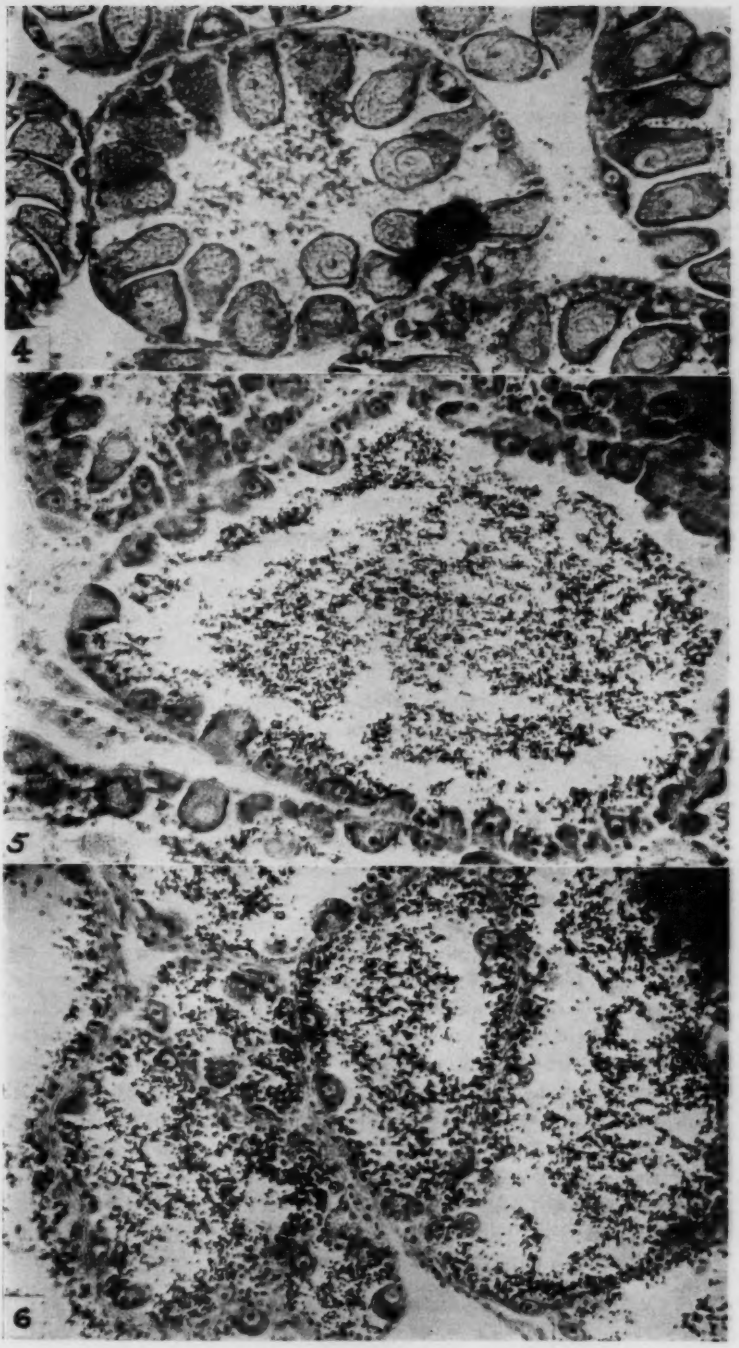


PLATE II

appear; Fig. 2 represents the female phase in which few or no spermatogonia can be certainly identified but presumably potential spermatogonia exist. They are, in fact, often clearly visible between the ovocytes of so-called females. It should be explained that the small nuclei which surround the eggs are not spermatogonia but belong to a chorion of nurse cells. They are much smaller than those of spermatogonia and apparently have no significance for this study.

Discussion of the Entire Life Cycle

We have not studied the very young teredos sufficiently to have a complete knowledge of all the details of this period of development. It appears, however, both from extended study of living animals and sections that an early male phase is dominant and characteristic as reported by Coe (1933), but this phase is of short duration. From the fifth to the sixth week after metamorphosis most individuals are in the active male phase but by the end of six weeks or two months many have transformed into functional females and have extruded considerable quantities of eggs as shown by the presence of embryos in the brood pouches. Although the male phase is normally of short duration and easily overlooked, there is reason to believe that some individuals become females without passing through the preliminary male phase. This applies particularly to those not very numerous individuals that extrude fertilizable eggs at six weeks of age and are therefore among the first to spawn.

It is apparent from our study of later stages that the female phase is of considerable duration. It normally covers a period of two or three months and includes two or three successive spawnings at monthly intervals. This female phase is in turn frequently followed by a second male phase which apparently persists to the end of the life of the individual. There is no apparent reason why there should not be a second female phase following upon the second male phase except that the duration of life of this species is from 12 to 15 months, which means that there are two spawning seasons at most. The noticeable increase in the ratio of males to females toward the end of adult life as shown by Table I, indicates that this sex inversion from female to male is taking place most actively from the middle to the end of the second breeding season (July and August). The sexual life cycle of these individuals would therefore be represented by: male-female-male. It is known from previous studies of *Teredo* (Grave, 1928) that young individuals which develop from eggs spawned late in the breeding season do not reach sexual maturity until the following summer. These individuals

have a single breeding season and their life cycle is represented by male-female.

The sexual cycle of individuals which become females without a preliminary male phase is not known because of the difficulty of distinguishing them from others of the population.

We have not had opportunity to verify Coe's evidence that individuals which pass through both a male and a female phase during the first season may become functional males at the opening of the second breeding season. (Coe, 1934.) These are no doubt among the large group that changes from male to female early in June and thus effectively reverses the sex ratio from greatly predominant male to almost equally predominant female within a period of one month. However, we have observed that sex inversion continues though in smaller numbers into July, and except for this group described by Coe (1934) the transformation to the second female phase takes place most actively in late June and July. Individuals which behave thus are presumably among the oldest of the over-wintering population and are one year old at the opening of the second breeding season. These favored individuals therefore have two breeding seasons. In this case, since we have shown that sex inversion is not confined to young individuals but takes place also in the adult animals, the sexual life cycle of this group whether inversion happens early in the second breeding season or later, should be represented by male-female-male-female.

On the factual side we have shown sexual inversion is taking place in fully grown *Teredo* during July and August both from female to male and from male to female. *Teredo navalis* therefore resembles the California oyster in its sexual life cycle rather than *Crepidula,* which has an early more or less persistent male phase which is followed by a permanent female phase. It is therefore properly called protandric whereas *Teredo* usually has one or more additional transformations to complete its sexual cycle.

Discussion and Conclusions

I

It may be observed that there is little difference between Professor Coe's data and ours except for the last two months of the life history of *Teredo.* This part of the life history deals with data and observations for July and August: of the second breeding season. What is the explanation of the final difference of interpretation?

In analysing Coe's data in support of the belief that the adult population is 10 per cent male after the middle of the breeding season, it should be pointed out that he draws his conclusion somewhat indirectly. Thus

in one case he analyses a population collected early in the summer and shows by a study of sections that it contains 10 per cent true males. The others are in the female phase or are clearly protandric females which should become functional females before the end of the breeding season, leaving 10 per cent permanent males (see Coe, 1933, p. 196). In another place (at the bottom of page 296) he used a group of 202 young teredos collected August 18–21. He shows that this group contains 18 males, which confirms the estimated ratio of 9 or 10 males to 100 females. Again on page 298, he uses a group of 220 young teredos for the same purpose. It would appear that these young animals of the current breeding season could not be confidently used in proving the existence of a certain sex ratio in adults. It is a difficult matter, it is true, to get reliable data on normal sex ratios at the end of the breeding season because so large a part of the population is dying of old age. It is evident, therefore, that the basis for the assumption that there are only 10 per cent males in adult populations of *Teredo* after the middle of the second breeding season rests upon indirect evidence which fails to take into account the continuation of sex inversion from female to male during this period as well as earlier. All of Coe's actual counts of adult animals collected during the second breeding season show not less than 30 or 35 per cent males, some of which are hermaphroditic or protandric females in the male phase of development. This group remains part of the population throughout the breeding season and is properly listed among the males for the reason that it consists of functional males producing active spermatozoa and no eggs. They frequently are devoid of distinguishable ovocytes.[1]

II

In conclusion, emphasis is given to two points which constitute the contribution of this paper to the study of sex inversion in Mollusca. First, males are without doubt much more abundant in *Teredo* than a survey of the literature indicates. They constitute from 30 to 50 per cent of adults and an even larger proportion of the young. Second, there is inversion of the sexes throughout the life of the *Teredo*. It is not confined to the very young individuals or to the opening of the second breeding season, as Coe has maintained in his papers on *Teredo*. The condition in *Teredo* is therefore strictly comparable to that of the

[1] The abundant males which appear after the middle of August, as Coe points out, are young animals of the current season between 2 and 4 cm. in length, whereas adult animals grown under favorable conditions reach a length between 20 and 30 cm. In any case, there is no necessity for confusing them. The young teredos do not appear in the population as breeding animals until the middle of August and are easily distinguished from the older ones. Our work has dealt only with adults.

gonads of the California oyster, *Ostrea lurida,* as described by Coe. Although the life of *Teredo* is short, covering not more than one and a half years (usually 12 to 14 months), the female after spawning two or three times at monthly intervals reverts rapidly to the male phase and produces abundant active spermatozoa. This is as much a characteristic of fully-grown teredos as of any other stage of their life history.

As to the cause of this inversion of the sexes, we have no theory to offer, but it seems to be due to genetic causes rather than to environmental influences.

BIBLIOGRAPHY [2]

Coe, W. R., 1932. Development of the gonads and the sequence of the sexual phases in the California oyster (Ostrea lurida). *Bull. Scripps Inst. Oceanography, Tech. Ser.,* **3**: 119.

Coe, W. R., 1933. Sexual phases in Teredo. *Biol. Bull.,* **65**: 283.

Coe, W. R., 1934. Sexual rhythm in the pelecypod mollusk Teredo. *Science,* **80**: 192.

Coe, W. R., 1935. Sexual phases in the prosobranch mollusks of the genus Crepidula. Science, **81**: 570.

Furrow, C. L., 1935. Development of the hermaphrodite genital organs of Valveta tricarinata. *Zeitschr. f. Zellforschung,* **22**: 282.

Gould, H. N., 1917. Studies on sex in the hermaphrodite mollusc (Crepidula planta). *Jour. Exper. Zoöl.,* **23**: 1, 225.

Grave, B. H., 1928. Natural history of the shipworm (Teredo navalis). *Biol. Bull.,* **55**: 260.

Nelson, T. C., 1922. The European pileworm. Circular No. 139, *N. J. Agr. Exp. Station.*

[2] Coe gives a more complete bibliography and review of the literature.

RELATION BETWEEN MATURATION DIVISION AND CLEAVAGE IN ARTIFICIALLY ACTIVATED EGGS OF URECHIS UNICINCTUS (VON DRASCHE)

YOSHI KUNI HIRAIWA AND TOSHIJIRO KAWAMURA

(From the Zoölogical Laboratory, Hiroshima University, Hiroshima, Japan)

Recently Tyler (1931*a*, 1931*b*, 1932*a*, 1932*b*) has reported interesting studies on the relation between the polar bodies and cleavage in the parthenogenesis of *Urechis caupo*. He observed that only the eggs which were incompletely activated and did not protrude polar bodies divided and formed embryos, when unfertilized eggs were treated with diluted sea water. The chromosomes seen at the first cleavage in such eggs took the appearance of tetrad and numbered haploid. He obtained high percentages of cleavage by suppressing the protrusion of polar bodies with double treatments of diluted sea water and thought that the chromosomes were divided into two groups first and then formed the cleavage nucleus, fusing with each other in this case, as judged from the cytological observation on the preparations.

The process of parthenogenesis in *Urechis unicinctus* manifested by our experiments differs remarkably from that in *Urechis caupo* and varies somewhat from those in *Thalassema* (Lefevre, 1907), *Chætopterus* (Allyn, 1912), *Amphitrite* (Scott, 1906) and *Cumingia* (Morris, 1917). We will discuss those points briefly.

Urechis unicinctus (von Drasche) is found abundantly at the seashore in the neighbourhood of our university and matures in winter. Experiments were done at room temperature, between 9°–21° C. The sea water used was previously heated to about 80° to sterilize sperms and was then left to be cooled to room temperature. It was aerated by stirring before using. When taking out the eggs from the mother animal, the body surface of the worm was washed with fresh water, and the segmental organs, which are filled with eggs, were also thoroughly washed with sperm-free sea water. Accordingly no eggs were activated by sperms in the control of the experiments of artificial parthenogenesis. As much care as possible was taken not to stimulate mechanically the unfertilized eggs in handling. Observations were made on living and fixed materials. Among the latter, besides permanent total and section preparations, temporary preparations stained by aceto-

carmine were used to determine the general behavior of the nucleus. Bouin's and Navashin's solutions were used as fixing reagents for total preparations and beside these Champy's solution was used for material to be sectioned. Before sectioning, the eggs were first attached with albumen to pieces of the thin sheath of toad liver or petals of *Chrysanthemum*, then dehydrated and imbedded in paraffin. The sections were cut at 10–25 μ. Heidenhain's iron hæmatoxylin was used for staining, and, in the case of the materials fixed by Champy's solution, they were bleached with H_2O_2; the affinity to dyes was intensified by Chura's method before staining.

The unfertilized eggs of *Urechis unicinctus* can be caused to develop artificially to various degrees by mechanical, physical, and chemical stimuli. When the eggs are put into sea water in a test-tube together with small pieces of wire net or cotton cloth and shaken lightly for 1–2 minutes, most of them are activated and finish maturation division. The maturation division is hardly distinguishable from that of fertilized eggs, except for a few eggs which show cytolysis. About 1 per cent of them cleave. Several of those which do not divide swim about, becoming ciliated larvæ later. If unfertilized eggs are exposed to warm sea water of about 31.5° C. for 10 to 30 minutes, some of them are activated, then cleave and become larvæ. Exposure to hypertonic sea water made by adding NaCl or KCl to normal sea water, causes almost all of the eggs to be activated. Some of them cleave and move about as swimming larvæ. The hypotonic sea water made by dilution with distilled water is also another agent which induces parthenogenesis. The best results were obtained by the use of ammoniacal sea water as a chemical reagent for stimulation. When exposed to a mixture of 5 cc. of 0.1 N NH_4OH and 100 cc. of normal sea water for 1 minute, most of the eggs are activated and finish maturation division. They never cleave as the result of such short treatment, but in the case of exposure for 30–40 minutes, 98–99 per cent show cleavage in the most favorable instances. Alkaline sea waters made by addition of NaOH or KOH are also effective reagents following treatment with NH_4OH sea water. Unfertilized eggs are also activated by KCN solution and several of them cleave once or twice. In the present studies we have chiefly used NH_4OH sea water as the stimulative agent for artificial parthenogenesis.

By examination of the relation between polar body protrusion and cleavage in living materials, though polar body protrusion is hindered by the stimulative agents to a certain degree, it is clear, as indicated in Table I, that there is no distinct correlation between it and cleavage, as was shown in the case of *Urechis caupo*. In the cases with one or two

polar bodies, it can not be claimed that they are of the same nature in each egg. When one polar body exists, it may be the first in some cases and the second in the others. Eggs with two polar bodies are classified into three groups, namely (1) those with a first and a second polar body, (2) those with two second polar bodies without any first one, and (3) those with two derived from the first polar body, with no second one. Eggs with three polar bodies resemble the fertilized eggs but such eggs are produced very rarely.

In order to clarify the correlation between the nature of the nucleus and the cleavage, we tried the following treatments with NH_4OH solution on the eggs. In the first place, the unfertilized eggs were made to protrude polar bodies, by stimulating them only to an extent in which maturation divisions were finished, but cell division was not induced. Then the capacities of those eggs to induce cleavage were examined by

TABLE I

Polar body formation and percentage of cleavage

Exp. No.	Room temperature	Agent	Length of exposure	Number of polar bodies			Percentage of cleavage
				0	1	2 or 3	
	°C.		min.	per cent	per cent	per cent	per cent
22*B*	15	$\frac{1}{10}$N NH_4OH, 5 cc. + sea-water, 100 cc.	45	12	9	9	99
26*B*	14	"	30	4	2	94	80
29*E*	20	"	30	2	48	50	89

the second treatment. Results of such double treatments are shown in Table II.

Cleavages which resulted from the above experiments become very irregular as compared with those from single treatments. The blastulæ formed by the cell divisions are composed of cells of various sizes and of abnormal forms.

It is maintained by Tyler (1932*a*) and many other workers that the spindle of the maturation division develops and becomes the cleavage spindle which induces the first cell division. Lefevre (1907) believed that when the first polar body is not formed in the eggs of *Thalassema*, two resting nuclei are produced abnormally and in most cases these two soon fuse to form the cleavage nucleus. Morris (1917) observed almost similar phenomena in the eggs of *Cumingia*, and Allyn (1912) thought that such phenomena may occur also in the eggs of *Chætopterus*.

Scott (1906) observed in *Amphitrite* that the dyads of chromosomes left in the egg as the result of first maturation division retreat to the center.

In the eggs of *Urechis unicinctus* various abnormalities take place in the maturation division and the protrusion of polar bodies is hindered as is shown in Table I, but transition of the spindle of first or second maturation division to the cleavage spindle is never seen. The maturation divisions induced by various artificial stimuli are almost alike in all eggs (irrespective of the protrusion or suppression of polar bodies) with regard to the structure of the tetrads, the uniformity of their number and the facts that they become monads by successive two divisions without any resting stage and that the resting nucleus is formed

TABLE II

Percentage of cleavage by second treatment

Length of exposure of second treatment	Stage of second treatment	
	Immediately after extrusion of first polar body	Immediately after extrusion of second polar body
min.	*per cent*	*per cent*
5	10	0
10	12	4
20	23	8
30	31	37
40	40	25

Exp. No. 36*A*. Room temperature 16° C. Agent of first treatment 1/10 N NH_4OH solution 2.5 cc. + sea water 100 cc. Time-length of first treatment, 8 minutes. Agent of second treatment, 1/10 N NH_4OH 5 cc. + sea water 100 cc.

Upon being returned to normal sea water after the first treatment, the formation of the first polar body was 100 per cent after 42 minutes and of the second one 100 per cent after 1 hour and 12 minutes, but no eggs cleaved later, when the eggs were not treated again.

after the second division. The numbers of monads left in the matured eggs vary depending on the number and nature of the polar bodies protruded. In the cases in which two polar bodies were protruded regularly as in fertilized eggs, monads of haploid number were left; when a second polar body was not formed, monads of diploid number left; when two second polar bodies were protruded originating from two groups of dyads placed in the egg on account of lack of first polar body formation, monads of diploid number were left; when only one second polar body was formed from one of the two groups of dyads above mentioned, monads of triploid number were left (Fig. 1); by the eggs with no polar body, monads of tetraploid number were left in the egg. When the distribution of the chromosomes on the spindle

at maturation division is irregular, the number of the chromosomes left in the egg becomes irregular in most cases (Fig. 2). There is one resting nucleus composed of monads in the eggs which have completed regular maturation division, as in fertilized eggs, but there are one to

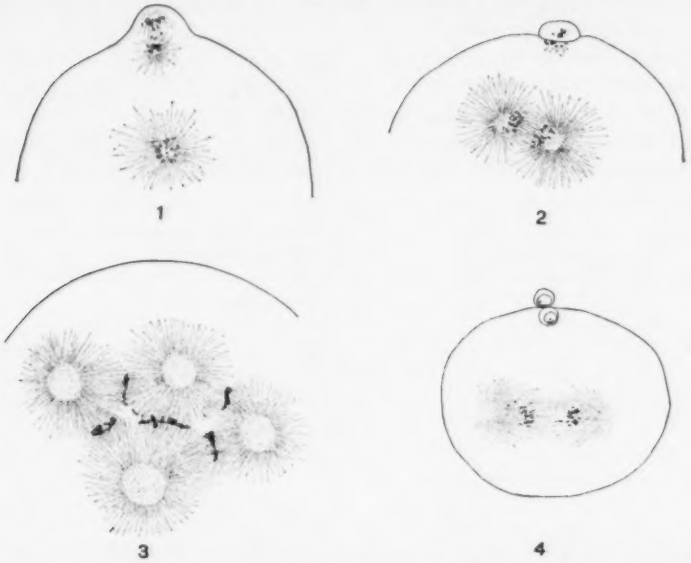


FIG. 1. Abnormal second maturation division in which only one second polar body was formed from one of the two groups of dyads. Another group of dyads divided deep in the egg simultaneously, but in this figure only one pole of it is shown.

FIG. 2. Irregular distribution of chromosomes at maturation division. Chromosomes seen just beneath the first polar body are those which are left in the egg at the first maturation division. The second maturation division took place deep in the egg.

FIG. 3. Tetraster produced at the time of first cleavage, when more than two nuclei exist and asters which have appeared in contact with the membrane of each nucleus connected each other. Chromosomes arranged on the equatorial plate are quite abnormal and irregular.

FIG. 4. Amphiaster produced at the time of first cleavage in the egg which was supposed to have finished maturation divisions similar to the fertilized egg. The aster and spindle are small in size and irregular as compared with normal ones.

four in other cases. Accordingly, it is almost impossible to infer the number of the chromosomes left in the matured eggs and the nature of the resting nucleus merely by the number of the polar bodies protruded.

The asters which appear at the first cleavage are always placed in contact with the nuclear membrane and the cytaster is never seen.

There are sometimes two asters, as in fertilized eggs, and these form an amphiaster; but besides the amphiaster, polycentric figures such as triaster or tetraster are found. Amphiasters are mostly produced in the eggs with one nucleus and when more than two nuclei exist, asters which have appeared in contact with the membrane of each nucleus connect with one another and show a polycentric figure (Fig. 3). In any case, the form and number of the chromosomes which have appeared are very irregular and indefinite. Even when an amphiaster is produced in the egg which is supposed to have finished maturation divisions similar to the fertilized egg, the chromosomes take on irregular granular, rod-like or thread-shaped forms. Their number is recognized as about haploid (17) in some cases but often exceeds it. The aster and spindle are generally small in size and irregular. At the anaphase almost equal numbers of chromosomes move to both poles (Fig. 4). When multipolar mitosis takes place, not only the numbers of chromosomes are indefinite but also their shapes are irregular. Of course at the metaphase extremely irregular division of chromosomes results. In any case eggs usually form two blastomeres of about equal size by the division of the cytoplasm but sometimes show irregular division or no cytoplasmic division.

Other eggs produce monasters at the first cleavage. Only the chromosomes divide but the division of the nucleus is not accompanied by that of the cytoplasm, though they invariably have complete maturation divisions almost similar to those of fertilized eggs and protrude both polar bodies. Monasters which appear at this time are roughly classified into two categories by the position, structure and size of the aster and the form and number of the chromosomes. (1) Astral rays are short and radiate surrounding the groups of chromosomes. Chromosomes are crooked and thread-like, as shown at the first cleavage of fertilized eggs, and are haploid in number. (2) Astral rays develop especially in one direction and irregular chromosomes of haploid or indefinite number are scattered widely on the astral rays. The relations between treatment, maturation division, and first cleavage are summarized as follows. Weak treatments cause the maturation divisions to complete in a manner similar to that in fertilized eggs and produce a monaster at the stage comparable to the first cleavage. Moreover, the shape of chromosomes which appear at this stage resembles those at first cleavage, and their number is about haploid. Agents of strength adequate to induce cleavage often interfere with the maturation division, produce various abnormalities, and also always injure the chromosomes in the first cleavage. The achromatic figures appearing at the first cleavage are monaster, amphiaster, triaster, or tetraster and in the cases

other than those with the monaster, chromosomal division is generally followed by cytoplasmic division.

In and after second cleavage the arrangement of blastomeres becomes very irregular, but eggs which have repeated divisions develop to blastulæ and few of them transform into gastrulæ and some trochophores. With the repetition of divisions, the shapes of chromosomes become constant in each cell. Among larvæ are found all stages ranging from almost normal to remarkably abnormal ones. A few of the trochophores lived for fifteen days and swam about in the water or on the bottom of the vessel with prototrochal cilia. The eggs which have repeated chromosomal division, but are not accompanied by cytoplasmic division, always show some differentiation of cytoplasm, and several of them swim about bearing cilia. In the studies of artificial parthenogenesis in Annelida and Mollusca, it is reported that the eggs of *Ophelia* (Bullot, 1904) and *Thalassema* (Lefevre, 1907) can not be differentiated without cleavage, but in many other animals such as *Amphitrite* (Scott, 1906), *Chætopterus* (Loeb, 1901; Lillie, 1902; Allyn, 1912), *Nereis* (Lillie, 1911; Just, 1915) and *Mactra* (Kostanecki, 1908, 1911) unicellular swimming larvæ are found. Such a larva of *Urechis unicinctus* has a ring of cilia quite similar to the prototroch at the equatorial position and swims with the comparatively long cilia.

BIBLIOGRAPHY

Allyn, H. M., 1912. The initiation of development in Chætopterus. *Biol. Bull.*, **24**: 21.

Bullot, G., 1904. Artificial parthenogenesis and regular segmentation in an annelid (Ophelia). *Arch. f. Entw.-Mech.*, **18**: 161.

Just, E. E., 1915. Initiation of development in Nereis. *Biol. Bull.*, **28**: 1.

Kostanecki, K., 1908. Zur Morphologie der künstlichen parthenogenetischen Entwicklung bei Mactra. Zugleich ein Beitrag zur Kenntnis der vielpoligen Mitose. *Arch. f. Mikr. Anat.*, **72**: 327.

Kostanecki, K., 1911. Über parthenogenetische Entwicklung der Eier von Mactra mit vorausgegangener oder unterbliebener Ausstossung der Richtungskörper. *Arch. f. Mikr. Anat.*, Abt. II, **78**: 1.

Lefevre, G., 1907. Artificial parthenogenesis in Thalassema mellita. *Jour. Exper. Zoöl.*, **4**: 91.

Lillie, F. R., 1902. Differentiation without cleavage in the egg of the annelid Chætopterus pergamentaceus. *Arch. f. Entw.-Mech.*, **14**: 477.

Lillie, F. R., 1911. Studies of fertilization in Nereis. *Jour. Morph.*, **22**: 361.

Loeb, J., 1901. Experiments on artificial parthenogenesis in annelids (Chætopterus) and the nature of the process of fertilization. *Am. Jour. Physiol.*, **4**: 423.

Morris, M., 1917. A cytological study of artificial parthenogenesis in Cumingia. *Jour. Exper. Zoöl.*, **22**: 1.

SCOTT, J. W., 1906. The morphology of the parthenogenetic development of Amphitrite. *Jour. Exper. Zoöl.*, **3**: 49.

TYLER, A., 1931*a*. The production of normal embryos by artificial parthenogenesis in the echiuroid, Urechis. *Biol. Bull.*, **60**: 187.

TYLER, A., 1931*b*. The relation between cleavage and total activation in artificially activated eggs of Urechis. *Biol. Bull.*, **61**: 45.

TYLER, A., 1932*a*. Chromosomes of artificially activated eggs of Urechis. *Biol. Bull.*, **63**: 212.

TYLER, A., 1932*b*. Production of cleavage by suppression of the polar bodies in artificially activated eggs of Urechis. *Biol. Bull.*, **63**: 218.

BIOLOGICAL MATERIALS

Since 1890 the Supply Department of the Marine Biological Laboratory has been furnishing both living and preserved specimens to schools and colleges. It is the desire of the Laboratory to continue this service in an efficient and satisfactory manner, and your coöperation is earnestly desired.

All our materials are freshly collected each season and are carefully prepared by men of long experience.

We have an excellent stock of specimens listed in our current catalogue for the spring term and summer school sessions.

We guarantee all our specimens to give absolute satisfaction.

Catalogues are furnished on request.

SUPPLY DEPARTMENT

Est. 1890

MARINE BIOLOGICAL LABORATORY

WOODS HOLE, MASS.

CONTENTS

Page

KLEINHOLZ, L. H.
Crustacean Eye-stalk Hormone and Retinal Pigment Migration 159

WHEELER, WILLIAM MORTON
Binary Anterior Ocelli in Ants 185

FISH, CHARLES J.
The Biology of Pseudocalanus minutus in the Gulf of Maine and Bay of Fundy 193

WELSH, JOHN H.
Diurnal Movements of the Eye Pigments of Anchistioides . . 217

GRAFFLIN, ALLAN L.
Renal Function in Marine Teleosts. III. The excretion of urea 228

SPARROW, F. K., JR.
Biological Observations on the Marine Fungi of Woods Hole Waters 236

LACKEY, JAMES B.
Occurrence and Distribution of the Marine Protozoan Species in the Woods Hole Area 264

GUTTMAN, S. A.
Effect of Ultraviolet Radiation on the Heart of Limulus polyphemus 279

WELSH, MARTHA F.
Oxygen Production by Zooxanthellae in a Bermudan Turbellarian 282

LAWSON, CHESTER A.
A Chromosome Study of the Aphid Macrosiphum solanifolii . 288

FULLER, JOHN L., AND GEORGE L. CLARKE
Further Experiments on the Feeding of Calanus finmarchicus . 308

TERROUX, KATHLEEN GODWIN
The Buffering Powers of Natural and Dialysed Helix pomatia Serum 321

GRAVE, B. H., AND JAY SMITH
Sex Inversion in Teredo navalis and its Relation to Sex Ratios 332

HIRAIWA, YOSHI KUNI, AND TOSHIJIRO KAWAMURA
Relation between Maturation Division and Cleavage in Artificially Activated Eggs of Urechis unicinctus (von Drasche) . . 344